Published in final edited form as:

Neurogastroenterol Motil. 2008 August ; 20(8): 891–899. doi:10.1111/j.1365-2982.2008.01124.x.

Relationship of cytochrome P450 pharmacogenetics to the effects of yohimbine on gastrointestinal transit and catecholamines in healthy subjects

A. E. Bharucha[★], T. Skaar[†], C. N. Andrews[★], M. Camilleri[★], S. Philips[†], B. Seide[★], D. Burton[★], K. Baxter[★], and A. R. Zinsmeister[‡]

[★]*Clinical and Enteric Neuroscience Translational and Epidemiological Research Program (C.E.N.T.E.R.), Mayo Clinic College of Medicine, Rochester, MN, USA*

[†]*Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, IN, USA*

[‡]*Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN, USA*

Abstract

Alpha-2 adrenergic receptors tonically inhibit colonic motility and the α_2 -adrenergic antagonist yohimbine, given intravenously, increased colonic tone in humans. However, the effect of yohimbine on colonic transit in humans is unknown. In this study, 30 healthy volunteers were randomized to yohimbine 16.2 mg p.o. t.i.d. or identical placebo for 7 days. We evaluated gastric emptying, small intestinal, and colonic transit by scintigraphy, bowel habits, haemodynamics and plasma catecholamines. As cytochrome P450 enzymes metabolize yohimbine, P450 genotypes (CYP2D6 and CYP3A4) were determined in 25 of 30 subjects who consented to genetic studies. The relationship between drug metabolizer status predicted by CYP2D6 and CYP3A4 and effects of yohimbine were assessed. Compared to placebo, yohimbine increased ($P \leq 0.02$) diastolic blood pressure, plasma noradrenaline concentrations and maximum tolerated volume during the satiation test [yohimbine (1241 ± 88 , mean $\pm$ SEM) vs placebo (1015 ± 87), $P = 0.054$]. However, yohimbine did not affect gastrointestinal transit. Based on CYP2D6 and CYP3A4 alleles, seven and 18 subjects were, respectively, extensive (EM) and poor (PM) metabolizers of yohimbine. Compared to EM, PM of yohimbine had a greater increase in plasma noradrenaline ($P = 0.1$ for PM vs EM), lower maximum tolerated volumes (1120 ± 95 vs 1484 ± 131 mL, $P = 0.02$), and faster colonic transit (i.e. GC₂₄ was 3.0 ± 0.4 vs 2.1 ± 0.5 , $P = 0.1$). These data suggest that CYP2D6 and CYP3A4 genotypes which determine the metabolism of yohimbine may influence its sympathetic and gastrointestinal effects.

Keywords

adrenergic; catecholamines; colon; cytochrome P450; gastrointestinal transit; yohimbine

INTRODUCTION

The sympathetic nervous system tonically inhibits colonic motility via presynaptic α_2 -adrenoreceptors, which inhibit acetylcholine release from neurons in the myenteric plexus and at the neuromuscular junction.^{1–3} The α_2 -adrenergic receptor antagonist yohimbine enhances

Address for correspondence Adil E. Bharucha MD, Clinical and Enteric Neuroscience Translational and Epidemiological Research Program (C.E.N.T.E.R.), Mayo Clinic, 200 First St. S.W., Rochester, MN 55905, USA. Tel: +1 507 538 5854; fax: +1 507 538 5820; e-mail: bharucha.adil@mayo.edu.

acetylcholine release from presynaptic terminals and thereby increases colonic tone,⁴ and induces colonic high-amplitude propagated contractions (HAPCs) in healthy subjects.⁵ Colonic HAPCs are associated with mass movement of colonic contents and precede defecation.^{6,7}

However, the effects of yohimbine on gastrointestinal and particularly colonic transit in humans have not been studied.

Yohimbine is metabolized by oxidation to both 11-hydroxy-yohimbine and 10-hydroxy-yohimbine.⁸ The inactive 10-hydroxy metabolite is highly water-soluble, rapidly excreted, and typically detected in urine but not in plasma. Although the 11-hydroxy metabolite has a 10-fold lower affinity for α_2 -adrenoreceptors, it has a similar capacity for blocking biological effects of stimulating α_2 -adrenoreceptors in cells perhaps because yohimbine is more highly bound to plasma proteins.⁹ However, there is considerable (i.e. more than 1000-fold) variation in the oxidation of intravenous yohimbine to 11-hydroxy-yohimbine in healthy subjects.¹⁰ Indeed, 17 of 152 subjects in that study did not oxidize yohimbine to 11-hydroxy-yohimbine and yohimbine augmented plasma noradrenaline to a greater extent in poor metabolizers.¹⁰ Moreover, impaired metabolism was inherited and predictable by specific isoforms at cytochrome P450 (i.e. *CYP2D6* and *CYP3A4* but not *CYP3A5*).

The aims of this study were first to assess the effects of yohimbine on gastrointestinal transit, satiation and plasma catecholamines in healthy subjects, and secondly, to ascertain if these effects were influenced by *CYP2D6* and *CYP3A4* genotypes that determine the metabolism of yohimbine.

METHODS

This was a double-blind, randomized, placebo-controlled, parallel-group study of the effects of yohimbine on gastrointestinal and colonic transit in 30 healthy subjects recruited by public advertisement. The study was approved by Mayo Clinic Institutional Review Board. Subjects recorded their bowel habits on a questionnaire for 7 days before (i.e. run-in period) and for 7 days during treatment with yohimbine (16.2 mg p.o. three times daily) or matched placebo (Fig. 1). Plasma catecholamines were measured before and during treatment. Gastrointestinal transit was assessed by scintigraphy during treatment.

Healthy subjects

All participants had an interview and a physical examination prior to enrolment. Exclusion criteria for controls included significant cardiovascular, respiratory, neurological, psychiatric or endocrine disease, irritable bowel syndrome,¹¹ anxiety or depression¹² as assessed by validated questionnaires, medications (with the exception of oral contraceptives or thyroid supplementation) and abdominal surgery (other than appendectomy or cholecystectomy). All females of child-bearing potential had to have a negative pregnancy test within 48 h of the study.

Drugs

Yohimbine (Spectrum Chemical Mfg Corporation, Gardena, CA, USA), an α_2 -adrenergic antagonist, is rapidly absorbed and eliminated after oral administration, with a mean elimination t_{half} of <1 h. More than 99% of yohimbine is cleared by hepatic metabolism, which produces major (i.e. 11-OH-yohimbine) and minor (i.e. 10-OH-yohimbine) metabolites.⁸ The only active metabolite of yohimbine (i.e. 11-OH-yohimbine),⁸ has an elimination half-life of approximately 6 h vs 1½ h for yohimbine. In previous studies, yohimbine given in doses up to 16.2 mg orally three times daily were well-tolerated and increased plasma catecholamines but

did not affect heart rate or blood pressure (BP) in normotensive subjects.¹³ Therefore, an oral dose of 16.2 mg orally three times daily was used in this study.

Scintigraphic transit: gastric emptying, small bowel and colonic transit

Gastrointestinal transit was measured by standard, validated scintigraphic methods.^{14,15} Gastric and small bowel transit were measured by ^{99m}Tc-labelled technetium (^{99m}Tc)-sulphur colloid two scrambled eggs, which were ingested with one slice of whole wheat bread and one glass of skim milk (300 kcal).^{14,15} ¹¹¹In labelled indium chloride (0.10 mCi) was mixed with a slurry of 5 mg activated charcoal to measure colonic transit.¹⁶ The slurry was evaporated to dryness on a hot plate at 90 °C, and the dried charcoal was placed into a size one gelatin capsule (Eli Lilly, Indianapolis, IN, USA) and coated with methacrylate (Eudragit S100; Degussa AG, Darmstadt, Germany), as in previous studies. A marker was placed on the patient's anterior superior iliac spine to map the location of the capsule in the colon. The capsule was given with a three-ounce glass of water.

We obtained abdominal images every hour for the first 6 h and at 10, 24 and 48 h. A variable region of interest programme was used to measure transit as in previous studies. The proportion of ^{99m}Tc reaching the colon at 6 h was used as a surrogate marker for small bowel transit. The primary summaries for comparison of transit profiles were: gastric emptying half-life time (t_{half}); colonic filling at 6 h; colonic geometric centre (GC) at 24 h; and ascending colon (AC) emptying t_{half} measurements based on geometric mean of counts in anterior and posterior AC regions of interest.

Data were analysed as in previous studies.^{14,15} The GC is the weighted average of counts in the different colonic regions [ascending (AC), transverse (TC), descending (DC) and rectosigmoid (RS)] and stool, respectively, 1–5. Thus, at any time, the proportion of colonic counts in each colonic region is multiplied by its weighting factor as follows:

$$(\%AC \times 1 + \%TC \times 2 + \%DC \times 3 + \%RS \times 4 + \%stool \times 5) / 100 = GC.$$

Ascending colonic emptying was summarized as t_{half} from linear interpolation of data on the AC emptying curve.

Daily stool diaries

Subjects recorded stool frequency, consistency (using the Bristol stool form scale), sense of evacuation, and ease of stool passage on daily bowel diaries during the run-in and treatment periods.^{17,18}

Satiation test

An adaptation of the method of Tack *et al.* was used. This is a meal-induced challenge test that provides information on the maximum tolerated volume (MTV) and the symptoms 30-min post-Ensure[®] challenge.¹⁹ Subjects were asked to ingest a nutrient drink (0.95 kcal mL⁻¹; ENSURE[®] Ross Laboratories, Abbott Park, IL, USA) from a cup through a straw, at a rate of 120 mL per 4 min. The cup was refilled and subjects were instructed to maintain intake at the required rate. At 5-min intervals, participants scored their fullness using a graphic rating scale that combined verbal descriptors on a scale graded 0–5 [0 = no symptoms; 1 = first sensation of fullness (threshold); 2 = mild; 3 = moderate; 4 = severe; 5 = maximum (unbearable fullness)]. Participants were asked to stop drinking when a fullness score of 5 was obtained, at which point the MTV was recorded.

Postprandial symptoms (bloating, fullness, nausea and pain) were measured 30 min after completing the test using a visual analogue scale (VAS) with 100-mm lines anchored with the

words 'none' (0 mm) and 'worst ever' (100 mm) at each end.²⁰ The aggregate symptom score was calculated as the sum of the four 100-mm VAS for each symptom (i.e. maximum 400).

Plasma catecholamines and haemodynamic parameters

Plasma catecholamines (i.e. plasma noradrenaline, adrenaline and dopamine) were assessed as indices of sympathetic activity once during the run-in period and twice during the treatment period (i.e. before and 1 h after taking yohimbine in the morning). Venous blood samples (10 mL) were collected in chilled glass tubes containing 0.05 mL of 10% sodium metabisulphite for catecholamines. Plasma was obtained by refrigerated centrifugation and fast-frozen in dry ice and acetone before storage at -70 °C until assayed. Catecholamines were extracted from plasma²¹ and subjected to HPLC with electrochemical detection.²² Blood pressure and heart rate were measured before and 2 h after medication.

Genotyping

Twenty-five of 30 subjects consented to provide blood for genotyping. Genomic deoxyribonucleic acid (DNA) was extracted from leucocytes in frozen, ethylenediaminetetraacetic acid-anticoagulated whole blood using a commercial kit (PureGene; Gentra Biosystems, Minneapolis, MN, USA); the typical yield was approximately 20 ng μL^{-1} blood. We characterized *CYP2D6* and *CYP3A4* genotypes, which have been shown to predict the metabolism of yohimbine.¹⁰

CYP2D6 The CYP allele designations refer to those defined by the CYP Allele Nomenclature Committee Genotyping. We used the CYP2D6 Tag-It multiplexed genotyping assay from Tm Biosciences (now part of Luminex Corporation, Austin, TX, USA). Genotyping was conducted according to the manufacture's instructions. This kit tests for 12 genetic variants (i.e. ★2A, ★3, ★4/10, ★5, ★6, ★7, ★8, ★9, ★12, ★2/17, the gene deletion and the gene amplification).²³ Subjects were categorized as extensive (i.e. two normal alleles or one normal and one reduced allele), intermediate (i.e. one normal and one inactive allele) or poor metabolizers (i.e. two inactive alleles) by inference from previous studies which compared genotype status to metabolism of drugs.^{24–27}

CYP3A4 The CYP3A4 single nucleotide polymorphism (rs#2246709; located in intron 7) was genotyped by a restriction fragment length polymorphism assay. The PCR was performed on 20 ng of genomic DNA using Eppendorf Hot Master Mix (Eppendorf North America, Inc, Westbury, NY, USA). The final concentrations of the primers were 200 nmol L^{-1} . A nested PCR reaction was used to obtain a small PCR fragment with a single *MnII* restriction site that differentiates between the wild-type and variant alleles. The primers for the first reaction were the following: FWD: GGAGTGTGATAGAAGGTGATCTAGTAGATC; REV: CACAATCTCATGGGATTTAGCAAAGG. This reaction amplified an 820-bp amplicon. This product was diluted 1 : 100 and used as a template for the second (nested) PCR reaction. The primers for the second reaction were the following: FWD: GCTCATACATTTTAGCTATCAGCC; REV: TCAGTAATCTATGTTTCATGCCAC. This reaction amplified a 252-bp amplicon. The 252-bp amplicon was digested with *MnII*, which cuts the variant allele, to produce a 102- and 150-bp fragment. The conditions for the PCR reaction were the following: the plates were cycled at 94 °C for 2 m; followed by 40 cycles of 94 °C for 30 s; and 60 °C for 30 s and 72 °C for 60 s.

Statistical analysis

The primary and secondary endpoints were analysed by an analysis of covariance adjusted for body mass index and gender; corresponding baseline values were also used when available. To follow an intent-to-treat paradigm, missing values in these analyses were imputed using the overall subjects mean value for the particular endpoint being assessed. A corresponding

adjustment in the error degrees of freedom (subtracting one for each missing value imputed) was made to adjust the residual error variance to account for this imputation. The relationship between CYP genotypes and endpoints (i.e. parameters of gastrointestinal transit, satiation and plasma catecholamines) was assessed by a similar analysis of variance in which the inferred metabolizer status (i.e. extensive or poor) and treatment group were used to predict the endpoints (i.e. haemodynamic parameters, plasma catecholamines and gastrointestinal transit). In these analyses, an interaction term for metabolizer status by treatment group was also included in each model to assess whether metabolizer status altered treatment effects. Metabolizer status could be classified in 25 of the 30 subjects who consented to participate in the genetic studies. While P -values <0.05 were considered statistically significant, P -values between 0.05 and 0.1 were considered to be of 'borderline significance'.

The sample size was estimated from previous studies in our laboratory, (mean GC_{24} for colonic transit 2.75, with a standard deviation of 1.0). Consequently, a sample size of 15 subjects per group, provided 80% power (with a two-sample t -test and two-sided α -level of 0.05) to identify a difference of 1.1 units in GC_{24} between placebo and yohimbine. This difference is considered to be clinically significant.^{28,29}

RESULTS

Gastrointestinal transit was assessed in 29 of 30 subjects enrolled in this study. One subject did not return for post-treatment assessments. Yohimbine was extremely well-tolerated and 24 of 30 subjects reported no side effects during the study. Only one of four subjects who reported transient increased anxiety or heart rate received placebo. One of two subjects who had epigastric burning and nausea received yohimbine. Baseline demographic characteristics, plasma catecholamines and haemodynamic parameters were comparable for both groups (Table 1).

Effects on gastrointestinal transit, satiation and bowel habits

Yohimbine did not significantly affect gastric emptying or colonic transit (Table 2). However, the GC for colonic transit at 24 h was >3.8 (i.e. the upper limit of normal in our laboratory) in three subjects who received yohimbine and two who received placebo. The effects of yohimbine on small intestinal transit (i.e. colonic filling at 6 h) were borderline significant. Yohimbine increased the MTV during the satiation test [yohimbine (1241 ± 88 , mean $\pm$ SEM) vs placebo (1015 ± 87), $P = 0.054$]. Symptom scores did not significantly differ between yohimbine and placebo (Table 3). Bowel diaries revealed that subjects who received yohimbine had more frequent ($P = 0.02$) bowel movements (i.e. 1.6 ± 0.11 stools per day) than placebo (i.e. 1.2 ± 0.1 per day). Stool consistency assessed by the Bristol stool form scale was higher ($P = 0.03$) for placebo (3.6 ± 0.2) than for yohimbine (2.9 ± 0.2). Scores for ease of passage and incomplete evacuation were not significantly different for yohimbine and placebo.

Effect on haemodynamic parameters and plasma catecholamines

Yohimbine increased diastolic BP ($P = 0.002$) (Table 4). During the treatment period, trough ($P < 0.01$) and peak plasma noradrenaline concentrations were higher for yohimbine than for placebo (Table 4). However, yohimbine did not have significant effects on plasma adrenaline or dopamine concentrations (data not shown).

Relationship between cytochrome P450 genotypes and pharmacodynamic effects

At *CYP2D6*, 18 of 25 subjects (72%) had two normal alleles (i.e. were extensive metabolizers), six had one abnormal allele (i.e. intermediate metabolizers) and one had two abnormal alleles (i.e. poor metabolizer) (Fig. 2). At the *CYP3A4* variant T16090C, six (24%) were wild-type homozygotes (G/G), eight (32%) were heterozygotes (G/A) and 11 (44%) were homozygotes

for the minor allele (A/A). Thereafter, the genotypes at both loci were combined to define subjects as extensive and poor metabolizers using data from a previous study which compared these genotypes to yohimbine metabolism in healthy subjects (Fig. 2).¹⁰ In that study, subjects who homozygous for the wild-type alleles at both loci (i.e. Wt/Wt at CYP2D6 and G/G at CYP3A4) and subjects who were heterozygous at both loci metabolized yohimbine most extensively of all genotype combinations. Therefore, in this study, subjects in these two genotype combinations were considered to be extensive metabolizers (i.e. seven subjects) while the remaining (i.e. 18) subjects were considered to be poor metabolizers. Nine of 18 subjects in the poor metabolizer group, and four of seven subjects in the extensive metabolizer group received yohimbine.

The effect of metabolizer status on treatment effects was assessed by overall gene by treatment interactions and thereafter by pairwise comparisons between poor and extensive metabolizers among subjects treated with yohimbine. These data suggest that the effects of yohimbine (vs placebo) on plasma noradrenaline concentrations, MTV and colonic transit were more pronounced in PM, as predicted by *CYP2D6* and *CYP3A4*; however, statistical tests were, in general, borderline significant (Table 5, Fig. 2). Indeed, all three subjects with abnormally rapid transit ($GC_{24} > 3.8$ at 24 h) in the yohimbine group were poor metabolizers while both subjects with rapid colonic transit in the placebo group were rapid metabolizers. In contrast, the metabolizer status predicted from CYP genotypes did not predict the haemodynamic response to yohimbine, gastric emptying, small intestinal transit, or ascending colonic emptying rate.

DISCUSSION

At the dose tested in this study, yohimbine does not affect satiation or gastrointestinal and colonic transit. However, metabolizer status predicted from CYP genotypes appears to influence the physiological effects of yohimbine. Thus, compared to rapid metabolizers, poor metabolizers randomized to yohimbine had a more pronounced augmentation in plasma noradrenaline, higher MTV and faster colonic transit. However, in some instances, these effects were of borderline statistical significance.

The sympathetic nervous system tonically inhibits colonic motility via adrenergic α_2 -receptors. Intravenous yohimbine increases colonic tone and tends to induce colonic HAPCs in healthy subjects.^{4,5,30} However, in this study, oral yohimbine did not affect gastric emptying or colonic transit and effects on small intestinal transit were of borderline significance. Oral yohimbine increased diastolic BP and plasma noradrenaline, indicative of biological effects. Moreover, the rise in plasma noradrenaline (i.e. 189 ± 23 – 377 ± 59 pg mL⁻¹) is comparable to the two- to three-fold increase in plasma noradrenaline after intravenous yohimbine at a dose (i.e. 0.125 mg kg⁻¹)³¹ known to increase colonic tone in healthy subjects.^{4,5,30} Nonetheless, it is possible that pharmacokinetic factors including variable bioavailability of oral yohimbine (i.e. 4.3–84.9%) and/or more extensive metabolism to 11-OH-yohimbine after oral than intravenous yohimbine may partly explain why yohimbine did not significantly affect colonic transit.^{8,13} Although 11-OH-yohimbine has 10-fold lower affinity for α_2 -adrenoreceptors than yohimbine, it has a similar capacity for blocking α_2 -adrenoreceptor-mediated effects on lipolysis and human platelet aggregation in cell systems, perhaps because the parent compound is more highly bound to plasma proteins.⁹ Pharmacodynamic mechanisms may also explain why yohimbine did not accelerate colonic transit. Thus, animal studies with yohimbine³ or after surgical removal of the sympathetic nervous system^{32,33} suggest that the contractile response to sympathetic blockade is brief and lasts only a few minutes. It is conceivable that a brief contractile response may not suffice to accelerate colonic transit and that acetylcholine released by α_2 -adrenergic blockade may inhibit further acetylcholine release via feedback mechanisms, thereby limiting the effects of α_2 -blockade.³ Another possible explanation for

the lack of an overall effect of yohimbine is that antagonism of central and peripheral α_2 -receptors results in potentially opposing consequences on gastrointestinal motility. Thus, net effects of yohimbine on colonic motor activity are dependent on the balance between potentially opposing consequences of its central and peripheral effects. Blockade of peripheral α_2 -adrenergic receptors (i.e. in the myenteric plexus and at the neuromuscular junction) would be anticipated to increase colonic motor activity, while centrally mediated increased sympathetic activity may reduce colonic motor activity. Further studies with peripherally selective α_2 -adrenergic antagonists are necessary to clarify this issue.

Yohimbine increased the MTV but did not affect symptom scores during the satiation test. These effects were more pronounced in poor compared to extensive metabolizers of yohimbine. While intravenous yohimbine did not affect fasting or postprandial gastric volumes, yohimbine augmented postprandial accommodation induced by glucagon-like peptide-1³⁴. Because peripheral α_2 -adrenergic effects would be anticipated to reduce gastric volumes, we suspect that yohimbine increased MTV by central effects.

Our results support a previous study demonstrating that pharmacogenetic factors (i.e. *CYP2D6* and *CYP3A4* genotypes) may influence the magnitude of yohimbine-induced increased sympathetic activity.¹⁰ Similar to intravenous yohimbine,¹⁰ oral yohimbine increased plasma noradrenaline concentrations to a greater extent in poor than in extensive metabolizers of yohimbine; however, effects were borderline significant. Moreover, our data suggest that *CYP2D6* and *CYP3A4* may also predict the effects of yohimbine on satiation and colonic transit; poor metabolizers had faster colonic transit (GC_{24}) but lower MTVs. Thus, the observed differences in the mean GC_{24} between poor and extensive metabolizers who received yohimbine (i.e. 0.9 units) were of borderline statistical significance but in the range considered to be clinically significant.^{28,29} Indeed, all three (of 15) subjects with abnormally rapid transit (i.e. colonic $GC_{24} > 3.8$) in the yohimbine group were poor metabolizers while both subjects with rapid colonic transit in the placebo group were rapid metabolizers. Perhaps the peripheral effects of higher concentrations of yohimbine, which has a higher affinity for α_2 -adrenoreceptors than 11-OH yohimbine, explains why the effects of yohimbine are more pronounced in poor than in extensive metabolizers. However, future studies with a larger sample size are necessary to confirm these findings. Of interest, the proportion of subjects who were inferred to be poor metabolizers in our exclusively caucasian participants [i.e. seven of 25 subjects (28%)] is comparable to that in a previous study (i.e. 18%).¹⁰

Because this dose of yohimbine did not accelerate colonic transit in healthy subjects, future studies should perhaps assess the effects of a higher dose in extensive and the same dose in poor metabolizers. Moreover, because the central effects of yohimbine, mediated via autonomic nerves, may retard transit, the peripheral effects of yohimbine may predominate, thereby accelerating transit in patients with autonomic neuropathy and constipation. Thus, the effects of yohimbine in constipated patients with an autonomic neuropathy should be assessed.

In conclusion, yohimbine at a dose of 16.2 mg orally three times daily increased plasma catecholamine concentrations indicating sympathetic stimulation but did not affect overall colonic transit. Yohimbine may induce a more pronounced sympathetic response and may accelerate overall colonic transit in subjects who are poor metabolizers, as characterized by *CYP2D6* and *CYP3A4* status.

ACKNOWLEDGMENTS

This study was supported in part by USPHS NIH Grant R01 DK068055, and by the Mayo CTSA grant M01-RR00585 from the National Institutes of Health in support of the Physiology Laboratory and Patient Care Cores.

REFERENCES

1. Bucknell A, Whitney B. A preliminary investigation of the pharmacology of the human isolated taenia coli preparation. *Br J Pharmacol* 1964;23:164–75.
2. Fishlock DJ, Parks AG. The action of nicotine on the circular muscle of the human ileum and colon in vitro. *Br J Pharmacol* 1966;26:79–86.
3. Gillis RA, Dias Souza J, Hicks KA, et al. Inhibitory control of proximal colonic motility by the sympathetic nervous system. *Am J Physiol* 1987;253:G531–9. [PubMed: 2889367]
4. Bharucha AE, Camilleri M, Zinsmeister AR, Hanson RB. Adrenergic modulation of human colonic motor and sensory function. *Am J Physiol* 1997;273:G997–1006. [PubMed: 9374695]
5. Malcolm A, Camilleri M. Coloanal motor coordination in association with high-amplitude colonic contractions after pharmacological stimulation. *Am J Gastroenterol* 2000;95:715–9. [PubMed: 10710063]
6. Bassotti G, Crowell MD, Whitehead WE. Contractile activity of the human colon: lessons from 24 hour studies. *Gut* 1993;34:129–33. [PubMed: 8432443]
7. Law NM, Bharucha AE, Undale AS, Zinsmeister AR. Cholinergic stimulation enhances colonic motor activity, transit, and sensation in humans. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G1228–37. [PubMed: 11668032]
8. Le Corre P, Dollo G, Chevanne F, Le Verge R. Biopharmaceutics and metabolism of yohimbine in humans. *Eur J Pharm Sci* 1999;9:79–84. [PubMed: 10494000]
9. Berlan M, Le Verge R, Galitzky J, Le Corre P. Alpha 2-adrenoceptor antagonist potencies of two hydroxylated metabolites of yohimbine. *Br J Pharmacol* 1993;108:927–32. [PubMed: 8097957]
10. Le Corre P, Parmer RJ, Kailasam MT, et al. Human sympathetic activation by alpha2-adrenergic blockade with yohimbine: bimodal, epistatic influence of cytochrome P450-mediated drug metabolism. *Clin Pharmacol Ther* 2004;76:139–53. [PubMed: 15289791]
11. Bharucha AE, Locke GR, Seide B, Zinsmeister AR. A new questionnaire for constipation and fecal incontinence. *Aliment Pharmacol Ther* 2004;20:355–64. [PubMed: 15274673]
12. Zigmond A, Snaith R. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361–70. [PubMed: 6880820]
13. Tam SW, Worcel M, Wyllie M. Yohimbine: a clinical review. *Pharmacol Ther* 2001;91:215–43. [PubMed: 11744068]
14. Camilleri M, Zinsmeister AR. Towards a relatively inexpensive, noninvasive, accurate test for colonic motility disorders. *Gastroenterology* 1992;103:36–42. [PubMed: 1612355]
15. Camilleri M, Zinsmeister AR, Greydanus MP, Brown ML, Proano M. Towards a less costly but accurate test of gastric emptying and small bowel transit. *Dig Dis Sci* 1991;36:609–15. [PubMed: 2022162]
16. Burton DD, Camilleri M, Mullan BP, et al. Colonic transit scintigraphy labeled activated charcoal compared with ion exchange pellets. *J Nuclear Med* 1997;38:1807–10.
17. Coulie B, Szarka LA, Camilleri M, et al. Recombinant human neurotrophic factors accelerate colonic transit and relieve constipation in humans. *Gastroenterology* 2000;119:41–50. [PubMed: 10889153]
18. Heaton KW, Ghosh S, Braddon FE. How bad are the symptoms and bowel dysfunction of patients with the irritable bowel syndrome? A prospective, controlled study with emphasis on stool form. *Gut* 1991;32:73–9. [PubMed: 1991641]
19. Chial HJ, Camilleri C, Delgado-Aros S, et al. A nutrient drink test to assess maximum tolerated volume and postprandial symptoms: effects of gender, body mass index and age in health. *Neurogastroenterol Motil* 2002;14:249–53. [PubMed: 12061909]
20. Delgado-Aros S, Camilleri M, Cremorini F, et al. Contributions of gastric volumes and gastric emptying to meal size and postmeal symptoms in functional dyspepsia. *Gastroenterology* 2004;127:1685–94. [PubMed: 15578506]
21. Eisenhofer G, Smolich JJ, Cox HS, Esler MD. Neuronal reuptake of norepinephrine and production of dihydroxyphenylglycol by cardiac sympathetic nerves in the anesthetized dog. *Circulation* 1991;84:1354–63. [PubMed: 1884458]

22. Holmes C, Eisenhofer G, Goldstein DS. Improved assay for plasma dihydroxyphenylacetic acid and other catechols using high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl* 1994;653:131–8. [PubMed: 8205240]
23. CYP2W1; Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee Ingelman-Sundberg M, Daly A, Knebert D W Available at: <http://www.cypalleles.ki.se/index.htm> (accessed on 17 March 2008).
24. Johansson I, Lundqvist E, Bertilsson L, Dahl M, LSSjoqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. [see comment]. *Proc Natl Acad Sci USA* 1993;90:118259 [PubMed: 7903454]
25. Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. [see comment]. *Am J Hum Genet* 1997;60:28495 [PubMed: 9012401]
26. Grieser EU, Zanger UM, Brudermanns U, et al. Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. *Pharmacogenetics* 1998;8:15–26. [PubMed: 9511177]
27. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3:229–43. [PubMed: 11972444]
28. Viramontes BE, Camilleri M, McKinzie S, Pardi DS, Burton D, Thomforde GM. Gender-related differences in slowing colonic transit by a 5-HT₃ antagonist in subjects with diarrhea-predominant irritable bowel syndrome. *Am J Gastroenterol* 2001;96:2671–6. [PubMed: 11569693]
29. Gonne J, Camilleri M, Ferber I, et al. Effect of alvimopan and codeine on gastrointestinal transit: a randomized controlled study. *Clin Gastroenterol Hepatol* 2005;3:784–91. [PubMed: 16234007]
30. Malcolm A, Camilleri M, Kost L, Burton DD, Fett SL, Zinsmeister AR. Towards identifying optimal doses for alpha-2 adrenergic modulation of colonic and rectal motor and sensory function. *Aliment Pharmacol Ther* 2000;14:783–93. [PubMed: 10848663]
31. Goldberg MR, Hollister AS, Robertson D. Influence of yohimbine on blood pressure, autonomic reflexes, and plasma catecholamines in humans. *Hypertension* 1983;5:772–8. [PubMed: 6352483]
32. Rostad H. Colonic motility in the cat. II. Extrinsic nervous control. *Acta Physiol Scand* 1973;89:91–103. [PubMed: 4761523]
33. Hulten L. Extrinsic nervous control of colonic motility and blood flow. An experimental study in the cat. *Acta Physiol Scand Suppl* 1969;335:1–116. [PubMed: 4315733]
34. Andrews CN, Bharucha AE, Camilleri C, et al. Effects of glucagon-like peptide-1 and sympathetic stimulation on gastric accommodation in humans. *Neurogastroenterol Motil* 2007;19:716–23. [PubMed: 17727392]

Figure 1. Study design. Subjects maintained a bowel diary throughout the 2-week study. Gastrointestinal transit, satiety and plasma catecholamines were assessed while subjects were being treated with yohimbine or placebo

Figure 2.

Distribution of cytochrome P450 (*CYP2D6* and *CYP3A4*) genotypes (left panel) and comparison of drug effects on plasma noradrenaline amongst genotypes (right panel). In the left panel, the numbers adjacent to the bars refer to the number of subjects who received yohimbine (Y) or placebo (P). There were no subjects in certain genotype combinations. The right panel shows peak plasma noradrenaline concentrations for subjects who received yohimbine. Plasma noradrenaline concentrations were the lowest among subjects who were extensive metabolizers. Based on a previous study, metabolizer status was predicted from *CYP450* genotypes. Plasma catecholamines were not available for one subject who was homozygous for the minor allele at *CYP3A4* and the major allele at *CYP2D6*.

Table 1

Demographic and baseline characteristics

Variable	Placebo (n = 15)	Yohimbine (n = 15)
Age (years)	29 ± 2	31 ± 2
No. females	13	12
BMI (kg m ⁻²)	23 ± 1	27 ± 1
Systolic BP (mmHg)	102 ± 3	109 ± 3
Diastolic BP (mmHg)	63 ± 2	66 ± 2
Heart rate (per minute)	64 ± 2	61 ± 2
Plasma noradrenaline (pg mL ⁻¹)	202 ± 28	211 ± 30
Plasma adrenaline (pg mL ⁻¹)	21 ± 2	35 ± 11
Plasma dopamine (pg mL ⁻¹)	12 ± 1	12 ± 1

BMI, body mass index; BP, blood pressure. All values except gender are actual mean values ± standard error (SEM).

Table 2

Effects of drugs on gastrointestinal transit

Parameter	Placebo (n = 15)	Yohimbine (n = 15)
Gastric emptying (t_{50} , min)★	138.3 ± 10.1	121.1 ± 10.2
Colonic filling (%) at 6 h†	29.0 ± 10.9	58.7 ± 9.9
Colonic transit (GC) at 24 h★	3.1 ± 0.3	2.7 ± 0.3
Ascending colonic emptying rate (t_{50} , hours)★	13.1 ± 2.1	13.1 ± 2.1

GC, geometric centre.

★Least squares mean values ± SEM adjusted for gender and BMI.

†Least squares mean values ± SEM adjusted for gender, BMI and an interaction term (i.e. gender and treatment).

Table 3

Effects of drugs on satiation

Parameter	Placebo	Yohimbine
Maximum tolerated volume (mL)★	1015.3 ± 86.7	1240.9 ± 87.7
Aggregated symptom score★ (maximum 400)	125.4 ± 21.0	135.8 ± 19.1
Nausea (maximum 100)†	16.5 ± 4.1	21.9 ± 8.1
Fullness (maximum 100)†	67.7 ± 4.0	73.4 ± 3.4
Bloating (maximum 100)†	33.3 ± 7.6	32.1 ± 7.2
Abdominal pain (maximum 100)†	14.1 ± 4.7	10.4 ± 3.8

★Least squares mean values ± SEM, adjusted for gender and BMI.

†Actual mean values ± standard error (SEM), $P = 0.054$ for treatment effect.

Table 4Effects of yohimbine on haemodynamic parameters and plasma catecholamines[★]

Parameter	Placebo (n = 15)	Yohimbine (n = 15)
Systolic BP postmedication (mmHg) [★]	113 ± 3	120 ± 4
Diastolic BP postmedication (mmHg) [★]	65 ± 2	72 ± 2 [†]
Heart rate postmedication (per minute) [‡]	66 ± 3	70 ± 3
Trough plasma noradrenaline (pg mL ⁻¹) ^{★§}	115 ± 37	253 ± 40 [‡]
Peak plasma noradrenaline (pg mL ⁻¹) ^{★§}	101 ± 42	368 ± 45 [‡]

[★]Least squares mean values ± SEM, adjusted for gender, BMI and baseline levels where appropriate.

[†] $P = 0.02$

[‡] $P \leq 0.01$, for treatment effect.

[§]Trough and peak plasma concentrations were measured immediately before and 1 h after medication respectively.

Table 5
Relationship between drug effects and metabolizer status

	Placebo (n = 12)		Yohimbine (n = 13)		P-value for gene by treatment interaction
	Poor metabolizers (n = 9)	Extensive metabolizers (n = 3)	Poor metabolizers (n = 9)	Extensive metabolizers (n = 4)	
Peak plasma noradrenaline (natural log, pg mL ⁻¹)	4.8 ± 0.1	5.0 ± 0.2	5.9 ± 0.1★	5.4 ± 0.2	0.08
Maximum tolerated volume (mL)	957 ± 95	1198 ± 143	1120 ± 95†	1484 ± 131	0.58
Gastric emptying (t ₅₀ , min)	138 ± 14	127 ± 21	123 ± 14	122 ± 19	0.8
GC ₄	0.7 ± 0.2	1.3 ± 0.3	0.9 ± 0.2‡	0.5 ± 0.3	0.04
GC ₂₄	3.0 ± 0.4	3.8 ± 0.6	3.0 ± 0.4★	2.1 ± 0.5	0.06
Ascending colonic emptying rate (t ₅₀ , hours)	14 ± 3	8 ± 4	12 ± 3§	16 ± 4	0.09

Least squares mean values ± SEM, adjusted for gender and BMI.

★P = 0.1,

‡P = 0.2,

§P = 0.3 vs extensive metabolizers treated with yohimbine.

†P = 0.01 for gene effects. While the gene by treatment interaction was not significant, P = 0.02 for poor vs extensive metabolizer status in the yohimbine group.