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Identification of serotonin 5-HT$_{1A}$ receptor partial agonists in ginger

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ABSTRACT

Animal studies suggest that ginger (*Zingiber officinale* Roscoe) reduces anxiety. In this study, bioactivity-guided fractionation of a ginger extract identified nine compounds that interact with the human serotonin 5-HT$_{1A}$ receptor with significant to moderate binding affinities (K_i = 3–20 μM). [^{35}S]-GTPγS assays indicated that 10-shogaol, 1-dehydro-6-gingerdione, and particularly the whole lipophilic ginger extract (K_i = 11.6 μg/ml) partially activate the 5-HT$_{1A}$ receptor (20–60% of maximal activation). In addition, the intestinal absorption of gingerols and shogaols was simulated and their interactions with P-glycoprotein were measured, suggesting a favourable pharmacokinetic profile for the 5-HT$_{1A}$ active compounds.

1. Introduction

The serotonin 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) is a G-protein coupled receptor widely expressed in the central nervous system (CNS), where it is involved in the modulation of mood and emotion and of different behavioural responses, including thermoregulation, sleep, feeding, aggression, and anxiety.[1–5] In addition, the 5HT$_{1A}$R is expressed on leukocytes in the spleen and gut where it may be involved in immunomodulatory processes.[6] Of all the serotonin receptor subtypes 5-HT$_{1A}$R is the dominant receptor responsible for anxiolysis and it is involved in the mechanism of action of several antidepressant drugs.[7] The therapeutically used azapirone anxiolytics buspirone, (Buspar®), gepirone (Gepiron®), and tandospirone (Sediel®) exert their activity mainly by partial agonism at the 5HT$_{1A}$R;[8] they may be equally effective in the therapy of depression as the selective serotonin reuptake inhibitors fluoxetine (Prozac®) and citalopram (Seropram®).[9] Furthermore, the 5-HT$_{1A}$R has been shown to play a role in the aetiology of migraine.[10]

The rhizome of ginger (*Zingiber officinale* Roscoe) is widely present in the general diet, but it is also used therapeutically to treat emesis and nausea,[11] migraine headache,[12] and several inflammatory diseases.[13,14] Quite intriguingly, ginger has also been reported to reduce anxiety in mice through as yet unknown mechanisms and ginger preparations are traditionally used to improve well-being and to treat mild forms of mental disharmony or migraine.[15,16] In addition, the US patent 5622704 claims that ginger rhizome is useful in the treatment of anxiety states, stating some in vivo evidence. In traditional Asian medicine ginger is used to treat fever and it has been reported that the ginger constituents 6-shogaol, 1-dehydro-6-gingerdione, 8- and 10-gingerol are able to attenuate serotonin-induced hypothermia in mice;[17] in combination with other plant extracts ginger was able to reduce the serotonin-mediated hyperphagic effect in mice.[18] Collectively, these reports suggest that the serotonergic system may be a target of ginger constituents, but only indirect effects of such compounds on 5-HT$_3$-triggered responses have been reported in the literature.[19,20] In contrast, no direct interactions between ginger constituents and 5-HT receptors have been demonstrated so far, despite the fact that the serotonergic system may be involved in the molecular mechanism of action of both the antiemetic and anxiolytic effects.

In the present study, we investigated whether secondary metabolites from ginger rhizome are able to functionally interact

Abbreviations: 5-HT$_{1A}$R, serotonin receptor 1A; 8-OH-DPAT, 8-hydroxy-2-(di-*N*-propylamino)-tetralin; CNS, central nervous system; GDP, guanosine-5′-*O*-diphosphate; GTP, guanosine-5′-*O*-triphosphate; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; HPLC, high-performance liquid chromatography; K_i, inhibition constant; Pgp, P-glycoprotein; TEER, trans-epithelial electrical resistance; TLC, thin layer chromatography.

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with the 5-HT_{1A}R. Moreover, we have assessed important pharmacokinetic parameters of the major bioactive ginger constituents. It has been reported previously that 6-gingerol is orally bioavailable,[21–23] but pharmacokinetic data on other lipophilic phenols from ginger are lacking. As we have found that some of these compounds can partially activate the 5HT_{1A}R, we have investigated the absorption of the major bioactive gingerols and shogaols in a Caco-2 model and we have assessed their effects on P-glycoprotein (Pgp) activity in vitro as Pgp transport is a limiting factor for CNS penetration.

2. Materials and methods

2.1. Chemicals and reagents

[^{3}H]-8-OH-DPAT (1 mCi/ml, 106–170 Ci/mmol), [^{35}S]-GTPγS (1 mCi/ml, 1250 Ci/mmol), [^{3}H]-GR65630 (1 mCi/ml, 77.2 Ci/mmol), [^{3}H]-CP55,940 (1 mCi/ml ,126 Ci/mmol), [^{3}H]-RTX (1 mCi/ml ,43 Ci/mmol), [^{3}H]FMLP, Ultima Gold scintillation cocktail, and human 5-HT_{1A}, 5-HT_3, FPRL-1, and CB_2 receptor overexpressing HEK-293 membranes were obtained from Perkin–Elmer, Switzerland. All silica gels were from Merck KGaA, Germany. Authentic standards (>95%) of 6-shogaol and 6-, 8-, and 10-gingerol were purchased from ChromaDex Inc., SantaAna, CA, USA. Rhodamine123 was purchased from Fluka, Switzerland. 5-HT_{1A}R transfected HeLa cells (clone HA-6[24]) were a gift from Novartis, Switzerland. CHO-K1 cells stably expressing TRPV1 were a gift from Dr. Zoltan Sandor, University of Debrecen, Hungary. Pgp (MDR1)-MDCK (Madin-Darby canine kidney) cells and the parental cell line were obtained from Dr. Stefanie Krämer, ETH Zürich, Switzerland. Solvents were obtained from Scharlau SA, Barcelona, Spain. All other chemicals were from Fluka Chemie AG, Switzerland or Sigma–Aldrich Chemie GmbH, Steinheim, Germany.

2.2. Plant material

The CO_2 extract of *Zingiber officinale* (Ginger Hot Flavor™ CO_2-to extract, Type Nr. 014.088, Ch. Nr. 120507) was provided by FLAVEX, Rehlingen, Germany. It is produced by supercritical fluid extraction of the rhizome with CO_2. According to the analysis certificate the extract contained 38.7% 6-, 8-, 10-gingerol, 4.3% 6-, 8-, 10-shogaol, 0.7% zingerone and 60 μl/g essential oil. The ginger essential oil used was a commercial grade water vapour distillate from Farfalla, Switzerland.

2.3. Caco-2 cell assay

The experiment was performed as published.[25] In brief, Caco-2 cells (ATCC, Rockville, MD) were grown at 37 °C, 5% CO_2 and humidified atmosphere according to ATCC recommendations and in the presence of 2 μg/ml amphothericin B.[25] Minimum essential medium eagle, non-essential amino acids, sodium pyruvate and amphotericin B were from Sigma–Aldrich Chemie GmbH, Steinheim, Germany, penicillin/streptomycin and glutamate were from Gibco Invitrogen, Switzerland, and foetal calf serum (heat inactivated for 30′ at 56 °C) was from Omnilab, Mettmenstetten, Switzerland. Cells were passaged at 80% confluency, seeded in Transwell-COL (6 well plate, 0.4 μm pore size; Corning Costar®, Cambridge, MA) at a density of 1×10^5 cells/ml and cultured for about three weeks to reach a trans-epithelial electrical resistance (TEER) exceeding 400 Ω/cm^2 determined at 37 °C using a Millicel-ERS system (MERS 000 01, Millipore). Transport studies were performed in Hanks Balanced Salt Solution.

2.4. Chromatography

18.9 g of extract were separated over silica gel by flash column chromatography (for a detailed account of the fractionation and isolation procedure see Supplementary data). All initial fractions were analyzed by thin layer chromatography (TLC, solvent system) and high-performance liquid chromatography. Fractions containing the same major product were combined, resulting in a total of 12 fractions labelled as 1–12 (Supplementary Fig. 1). Fractions 2–10 were tested in the radioligand displacement assay. The detailed HPLC method is described in Supplementary Table 1.

2.5. Displacement assay

The radioligand displacement assay was performed as reported previously.[26,27] For details see Supplementary data.

2.6. 5HT_{1A}R functional assays

The functional assays with GTPγS in 5-HT_{1A}R transfected HeLa cells were performed as follows: sub-confluent cells were re-suspended and vortexed in ice-cold buffer A (50 mM TRIS, 200 mM NaCl, 10 mM EGTA, 3 mM EDTA, and 0.1% Sigma protease inhibitor, adjusted to pH 7.6 with HCl at 4 °C) followed by centrifugation at 40,000g for 25 min at 4 °C. The pellet was washed with buffer B (50 mM TRIS, 200 mM NaCl, 1 mM EGTA, 0.3 mM EDTA, and 0.01% Sigma protease inhibitor cocktail P8340, adjusted to pH 7.6 with HCl, at 4 °C), briefly sonicated and homogenized with a dounce homogenizer, followed by centrifugation at 40,000g for 25 min at 4 °C. The pellet was re-suspended in buffer C (50 mM TRIS, 100 mM NaCl, 0.3 mM EDTA, 10 mM Mg(OAc)2, 0.2 mM 1,4-dithiotreitol (DTT), and 10% sucrose, adjusted to pH 7.6 with HCl, at 4 °C) to result in a 1.5–3 mg/ml protein concentration. Cells were stored at −80 °C until used.

2.5 μl membrane preparation was mixed with 96.5 μl incubation buffer (20 mM MOPS, 10 mM Mg(OAc)2, 30 μM GDP, 0.2 mM DTT, adjusted to pH 7.4 with KOH, at 30 °C) containing cold GTPγS (as the tetra-lithium salt) and incubated for 20 min at rt. The mixture was then cooled to 4 °C for 15 min and measurement was started by addition of 1 μl hot GTPγS in incubation buffer (final concentration of 98 pM cold and 2 pM hot GTPγS). After 30 min at 30 °C the reaction was stopped by cooling to 4 °C followed by filtration over Whatmann GF/B glass fibre filter (pre-soaked with wash buffer containing 1 mM ATP) and washed three times wash buffer (20 mM HEPES, 10 mM Mg(OAc)2, 10 μM ATP, 0.2 mM DTT, adjusted to pH 7.4 with KOH, at 4 °C. Filters were shaken in 3 ml scintillation cocktail until complete disintegration on a shaker KS10, Edmund Bühler, Tübingen, Germany, and then measured with a Beckman LS 6500 Multi-Purpose Scintillation Counter.

Non-specific binding was determined with 100 μM cold GTPγS and 100% activity with 10 μM 8-OH-DPAT. Test compounds were stored as 5 mM stock solutions in DMSO or in GDP-free incubation buffer at −80 °C. All experiments were repeated at least three times, using different membrane preparations.

2.7. Rhodamine123 efflux assay in Pgp transfected MDCK cells

Pgp activity was determined by Rhodamine123 (Rh123)-efflux, a fluorescent Pgp substrate, using human MDR-1 (Pgp) transfected MDCK cells (kindly obtained from Dr. Stefanie Krämer, ETH Zurich, Switzerland). Briefly, cells (1×10^6 cells/tube) were incubated with Rh123 (Sigma, St. Louis, USA) at a final concentration equal to 500 μM for 20 min at 37 °C. Following centrifugation, cells were washed and incubated with Rh123-free medium, in the absence or presence of test compounds at final concentrations of 10 and 30 μM for 1.5 h, at 37 °C. The fluorescence was measured using a

FACScan (Beckton Dickinson, USA) and the CellQuest software. Verapamil and vinblastine (both from Fluka, Switzerland) were used as positive controls. To validate the assay, untransfected MDCK cells were used which showed significantly less efflux of Rh123 over 1.5 h.

2.8. Statistical analysis

Results are expressed as mean values ± SD or SEM (depending on experiment) for each examined group. Statistical significance of differences between groups was determined by the Student's *t*-test (paired *t*-test) with GraphPad Prism software. Outliners in a series of identical experiments were determined by Grubb's test (ESD method) with α set to 0.05. Statistical differences between treated and vehicle control groups were determined by Student's *t*-test for dependent samples. Differences between the analyzed samples were considered as significant if $p \leqslant 0.05$.

3. Results

3.1. Bioactivity-guided isolation of 5-HT$_{1A}$ receptor ligands from ginger

Based on the hypothesis that certain ginger constituents may be able to modulate the 5-HT$_{1A}$R (vide supra), the commercial food-grade ginger CO_2 Hot Flavor™ ginger extract was tested for displacement of the high-affinity 5-HT$_{1A}$R-selective ligand [^{3}H]-8-OH-DPAT and the 5-HT$_3$R-selective ligand [^{3}H]-GR65630 from membranes obtained from HEK-293 cells transfected with serotonin 5-HT$_{1A}$ and 5-HT$_3$ receptors, respectively. In agreement with a previous report,[20] the ginger extract tested did not show a significant displacement (>50% at 25 μg/ml) of the radioligand from the 5-HT$_3$ cation channel (data not shown). In contrast, the extract and major fractions thereof strongly displaced [^{3}H]-8-OH-DPAT from the 5-HT$_{1A}$R (Fig. 1A), with a K_i value for the whole extract of 11.57 ± 1.56 μg/ml (Fig. 1B and C). As shown in Figure 2, 25 μg/ml of the ginger extract also displaced the high-affinity ligand [^{3}H]RTX (resiniferatoxin) from the vanilloid receptor transient receptor potential V1 (TRPV1) (approximately 50% displacement), a calcium channel already known to be a target for the spicy gingerols and shogaols.[28] In contrast, it did not significantly displace [^{3}H]CP55,940 from the cannabinoid type-2 receptor (CB_2) or [^{3}H]FMLP from the formyl peptide like-1 receptor (FPRL1) (Fig. 2), both of which are GPCRs. Ginger essential oil did not displace the radioligand (not shown), thus excluding non-specific lipid membrane effects of monoterpenes that are also present in the extract. Although limited to a few selected cases, these data indicated that the ginger extract did not bind indiscriminately to just any membrane-embedded receptor and this finding provided the impetus for a more detailed investigation of the interactions of individual ginger constituents with the 5-HT$_{1A}$R.

For this purpose we undertook an iterative bioactivity-guided fractionation of the CO_2 ginger extract employing flash liquid chromatography (FC) and high-performance liquid chromatography (HPLC) (Supplementary Fig. 1), which finally led to the isolation of 9 compounds exhibiting significant displacement of [^{3}H]-8-OH-DPAT from the 5-HT$_{1A}$ R (>50% displacement at 25 μg/ml). These compounds (Table 1) were characterized by spectroscopic and spectrometric means, including 2D and 3D nuclear magnetic resonance (NMR) measurements and mass spectrometry (ESI-MS); they were also compared to commercial samples, if available. Based on the K_i values obtained for all major compounds in the radioactive ligand displacement assay (Table 1), 6-gingerol does not bind to the 5-HT$_{1A}$R with any measurable affinity, while K_i values between 3 and 20 mM were determined for all other compounds. Overall, binding appears to correlate with the length of the aliphatic tail, with longer chains leading to higher affinity and 10-gingerol, 10-shogaol and 1-dehydro-10-gingerdione (1-DH-6-GDO) being the strongest binders (Table 1).

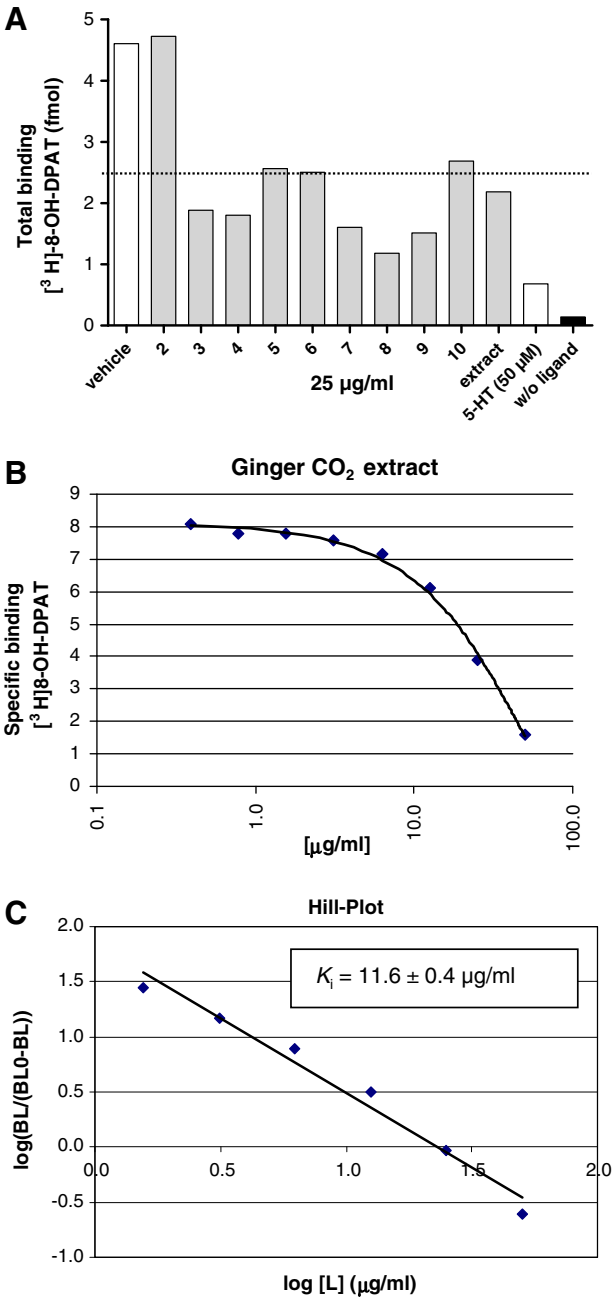


Figure 1. (A) Displacement of [^{3}H]-8-OH-DPAT by the ginger Hot Flavor™ CO_2 extract (extract) and chromatographic fractions (2–10). Serotonin (5-HT) was used as a positive control (50 μm), (B) concentration-dependent displacement of [^{3}H]-8-OH-DPAT by ginger Hot Flavor™ CO_2 extract and corresponding inhibition constant (K_i value) obtained, (C) linearized data in Hill plot. Data show mean values of 3 independent experiments.

3.2. 10-shogaol and 1-dehydro-6-gingerdione are 5-HT$_{1A}$ receptor partial agonists

In order to address the question whether the ginger extract and its 5-HT$_{1A}$R binding constituents are able to functionally modulate the 5-HT$_{1A}$R, 1–50 μg/ml of the ginger extract and 10 μM of each compound were analyzed in [^{35}S]-GTPγS assays,

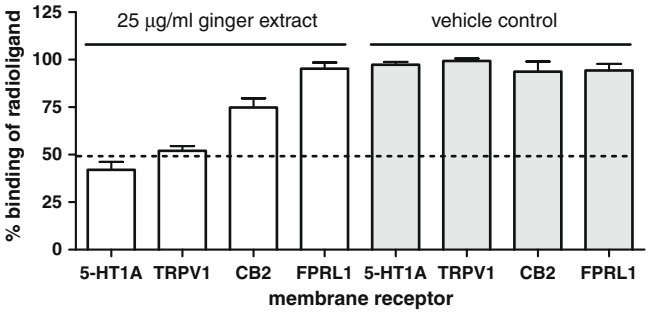


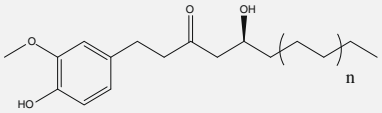
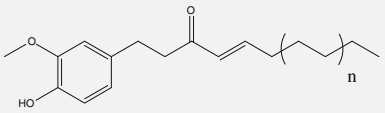
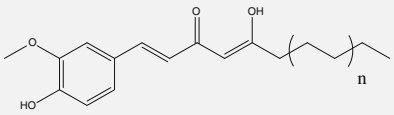
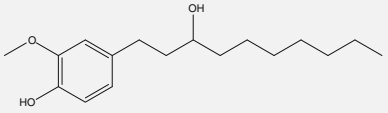
Figure 2. Displacement of selective radioligands from different membrane proteins (transient receptor potential vanilloid-1; TRPV1, cannabinoid type-2; CB_2; formyl-peptide like-1 receptor; FPRL-1) by ginger Hot Flavor™ CO_2 extract (25 µg/ml), Displacement of radioligands was observed from $5HT_{1A}$ and TRPV1, but not from CB_2 or FPRL-1. Data are mean values of 3 independent experiments ± S.D.

both alone and in combination with DPAT and serotonin. As expected, in this assay 10 µM of serotonin, which yielded a K_i value <50 nM in the displacement assay, induced approximately 100% of the maximal effect on [^{35}S]-GTPγS binding that is achievable with 8-OH-DPAT (Fig. 3). The ginger extract potently increased [^{35}S]-GTPγS binding (approximately 40% of the maximum effect of DPAT) at concentrations as low as 10 µg/ml, while 50 µg/ml led to membrane-toxic effects and the disruption of [^{35}S]-GTPγS binding (Fig. 3). For the individual ginger constituents effects >10% relative to the maximal effect induced by DPAT (100%) were only observed with 10-gingerol, 6-dihydroparadol, 10-shogaol, and 1-DH-6-GDO (Fig. 3). The two latter compounds showed partial agonism (>20%) and where therefore subjected to a concentration-effect analysis (Fig. 4). Unfortunately, these experiments were hampered by the fact that 10-shogaol and 1-DH-6-GDO apparently altered membrane integrity in a non-specific way, thus leading to the disappearance of [^{35}S]-GTPγS binding at concentrations above 20 µM (Fig. 4). Consequently, the maximum effects remain unknown for 10-shogaol and 1-DH-6-GDO and it was not possible in this assay format to differentiate between partial and full agonism. 8-, and 10-gingerol, 8-shogaol, and 6-dihydroparadol all behaved as weak partial agonists, while 6-shogaol, 1-DH-8-GDO, and 1-DH-10-GDO did not show any intrinsic activity. None of the compounds tested was able to significantly modulate [^{35}S]-GTPγS binding induced by DPAT or serotonin (data not shown). Intriguingly, the whole ginger extract not only induced a concentration-dependent activation of the $5\text{-}HT_{1A}R$, but the magnitude of the effect was significantly more pronounced than for any of its individual constituents (at the same w/v concentration), reaching as much as 60% [^{35}S]-GTPγS binding (relative to the maximum DPAT effect) at a concentration as low as 20 µg/ml (Fig. 4). This clearly suggests that the different components of the whole ginger extract may act in an additive or even super-additive manner at a functional level.

3.3. Passive diffusion of gingerols and shogaols across Caco-2 monolayers

To gain some basic insight into the pharmacokinetic behaviour of these compounds we next investigated whether the major gingerols and shogaols can passively diffuse through a monolayer of human adenocarcinoma colon epithelial cells (Caco-2 cells), an in vitro cell culture system widely used to mimic the intestinal lining.[25] The ginger CO_2 Hot Flavor™ extract (50 µg/ml) was added to either the apical or the basolateral side of the monolayer and transport of compounds through the monolayer was analyzed in both configurations, employing an HPLC method that was developed to quantify gingerol-type constituents from cell culture medium at sufficiently high resolution. The maximal non-cytotoxic concentration in this assay format was found to be 50 µg/ml and the integrity of the monolayer was monitored throughout the experiment by visual inspection and by measuring trans-epithelial electrical resistance (TEER; >400 Ω/cm^2). 6-, 8-, 10-gingerol and 10-shogaol crossed the Caco-2 monolayer by slow passive diffusion (10–30% after 3 h) while in this setup approximately 50% was retained in the cellular monolayer, which was isolated and extracted (Fig. 5). Under the experimental conditions used, 6-gingerol diffused across the monolayer with significantly higher efficiency than its dehydrated counterpart 6-shogaol (Fig. 5), in spite of it higher polarity. However, the incubation time in our assay was limited by both membrane effects and the sensitivity of the HPLC method.

Table 1
Binding affinities to the $5\text{-}HT_{1A}R$

Chemical structure	Extracts and isolated compounds	*Ki* ± S.D. (µM)	*Ki* ± S.D. (µg/ml)
	Ginger CO2 Hot Flavor™ extract		11.57 ± 1.56
	Ginger essential oil		>50
	6-Gingerol (*n* = 1)	>100	>50
	8-Gingerol (*n* = 2)	11.71 ± 4.35	3.77
	10-Gingerol (*n* = 3)	3.74 ± 1.77	1.31
	6-Shogaol (*n* = 1)	18.02 ± 4.77	4.97
	8-Shogaol (*n* = 2)	13.05 ± 1.19	3.97
	10-Shogaol (*n* = 3)	5.84 ± 1.14	1.94
	1-Dehydro-6-gingerdione (*n* = 1)	6.53 ± 0.72	1.89
	1-Dehydro-8-gingerdione (*n* = 2)	6.54 ± 1.35	2.08
	1-Dehydro-10-gingerdione (*n* = 3)	3.59 ± 1.08	1.24
	6-Dihydroparadol	9.53 ± 0.70	2.67

Inhibition constant (K_i) values were determined by displacement of [^{3}H]-8-OH-DPAT from receptor-transfected HEK-293 cells (see Supplementary methods). Data were obtained in at least 3 independent experiments. In this assay the K_i value for serotonin was <50 nM.

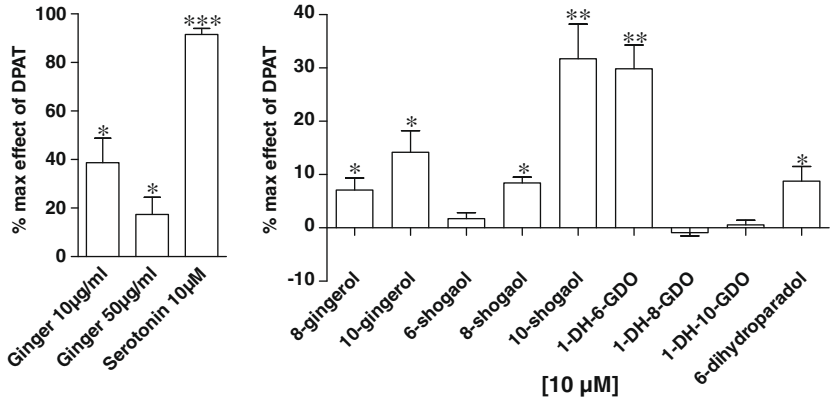


Figure 3. Effect of ginger extracts and ginger-derived isolated $5HT_{1A}$ ligands on G_i activation in $5HT_{1A}$ R transfected HEK-293 cells as assessed by [^{35}S]GTPγS binding. Activation efficiency is relative to the maximum effect achievable with 8-OH-DPAT (8-hydroxy-di-propylaminotetralin); DH-DGO = dihydrogingerdione. Data are mean values of at least three independent experiments using three membrane preparations (±S.D). $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$; $^{***}P \leqslant 0.005$.

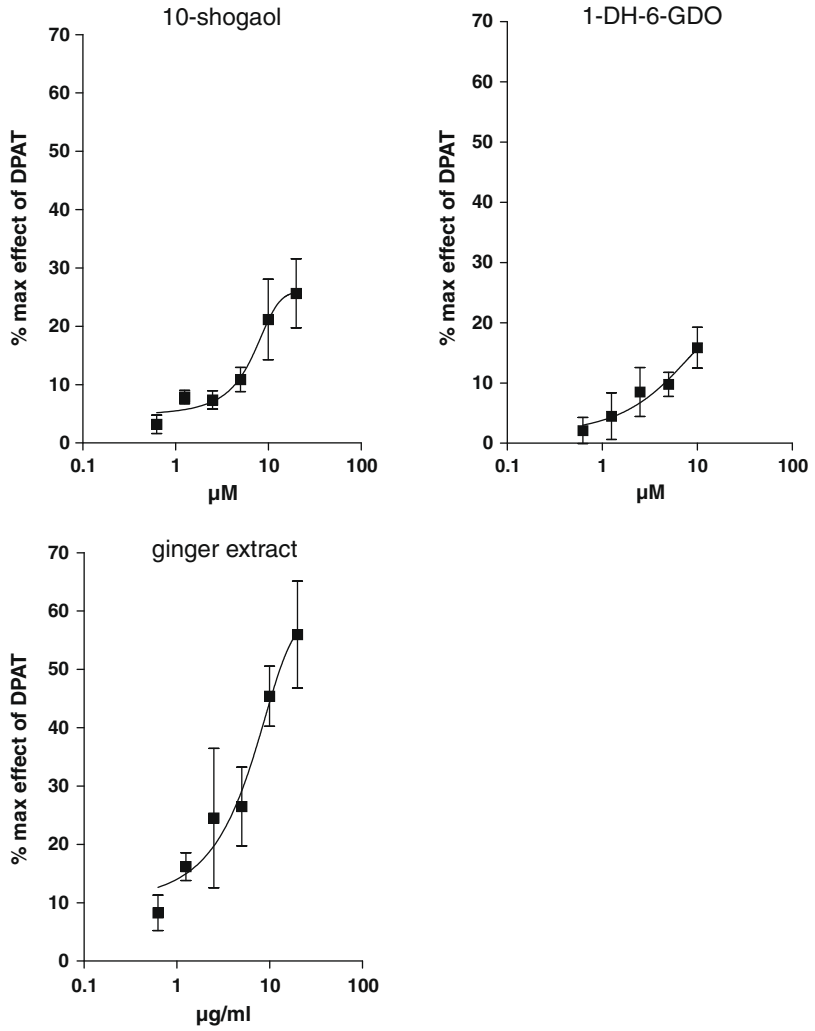


Figure 4. Functional effects in 5-HT_{1A} R transfected HEK-293 cells. Concentration-dependent action on [^{3}H]GTPγS binding: (A) 10-shogaol, (B) 1-dehydro-6-gingerdione (1-DH-6-GDO). At concentrations above 20 µM the assay became dysfunctional. Data obtained with concentrations >20 µM could therefore be misleading and the maximum effects of the compounds cannot not be determined. The most potent induction of [^{3}H]GTPγS binding was observed for the Hot Flavor™ CO_2 ginger extract, which led to 60% activation at the highest concentration tested. Data are mean values of 3 independent experiments ± S.D.

3.4. Interaction of gingerols and shogaols with human P-glycoprotein

Since P-glycoprotein (Pgp) substrates generally show a limited passage across the blood brain barrier we assessed whether gingerols and shogaols were able to competitively inhibit the efflux of rhodamine123 in human Pgp transfected MDCK cells. Neither gingerols nor shogaols either alone or in combination were found to act as substrates for Pgp up to a concentration up to 30 µM (Fig. 6). Unlike the positive controls verapamil (which is used as

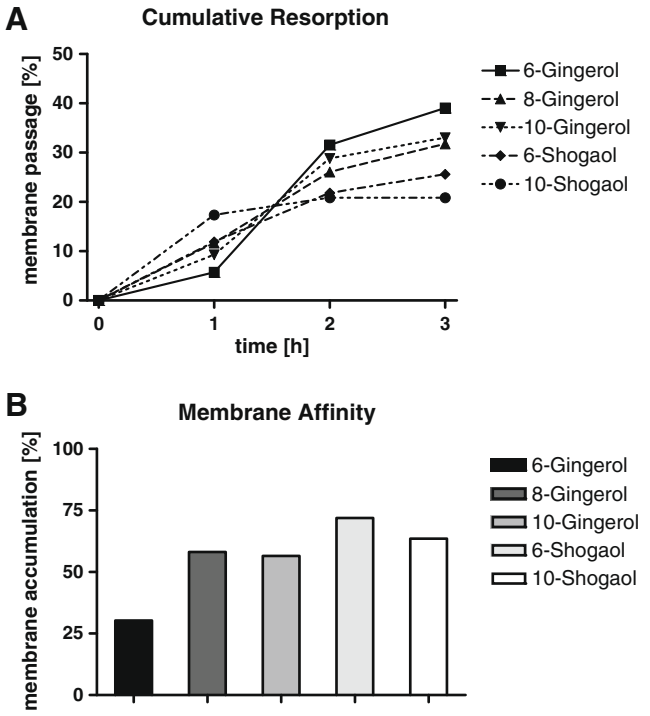


Figure 5. (A) Time-dependent passage of major ginger constituents (50 μg/ml) across Caco-2 monolayers (from apical to basolateral). The passage in both directions is equal (data not shown) and compounds are likely to move by passive diffusion, (B) accumulation of compounds in cell membranes was high, except for 6-gingerol, and inversely correlates with absolute compound concentration in the applied extract. Shown are mean values; S.D. was less than 15%.

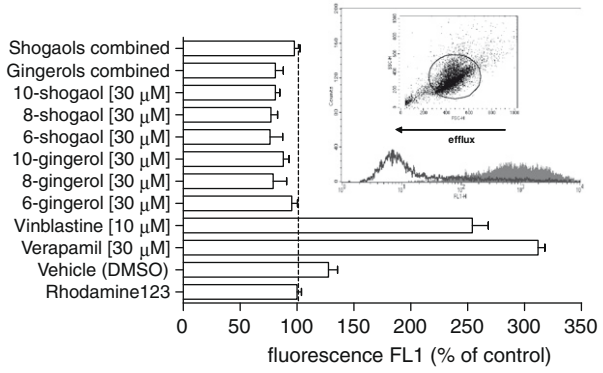


Figure 6. Effects of shogaols and gingerols on Pgp-mediated rhodamine123 (10 μM) efflux from MDCK cells (2×10^6 cells) stably transfected with human MDR-1 (Pgp). Shown is the mean fluorescence measured (geo mean in FACS, 10^4 cells counted, see histogram insert) as % of remaining cellular rhodamine123 after washing, relative to control. The Pgp inhibitor verapamil and the Pgp substrated vinblastine were used as positive controls. Data show mean values of 3 independent experiments ± S.D.

a Pgp inhibitor) or vinblastine (a classical Pgp substrate) the ginger compounds did not inhibit rhodamine123 efflux. The slightly increased efflux observed (Fig. 6) is likely due to non-specific membrane effects of the lipophilic gingerols and shogaols.

4. Discussion

Our study establishes a molecular interaction between ginger constituents and the serotonin 5-HT_{1A}R and thus provides a first rationale for the anxiolytic and mood modulating effects of ginger, as well as the anti-emetic properties of certain ginger-derived secondary metabolites, which may be mediated by partial agonism at the 5-HT_{1A}R. Importantly, ginger is considered to be safe and dietary intake of fresh ginger rhizome can easily exceed 5 g (average daily consumption in India or in Sushi cuisine); physiological concentrations of ginger constituents in the μM range may thus be achievable, at least in theory. We have obtained data from a Caco-2 monolayer absorption model which underpin existing pharmacokinetic data for the bioactive gingerols in mice.[16] Based on the finding that the main ginger constituents do not functionally interfere with Pgp (i.e., are no Pgp substrates or inhibitors), it is well conceivable that these lipophilic compounds could reach the CNS and this possibility should be addressed in future studies. In particular, it still needs to be investigated whether brain concentrations can be achieved that would be sufficient to trigger 5-HT_{1A}R-mediated pharmacological effects.

Given that prevention and alternative medicine enjoy increasing popularity and that anxiety disorders are very common in societies of developed countries and often affect young people[29] consumption of ginger rhizome, its extracts, or of 10-shogaol could be a cheap, safe, and efficient way of addressing milder and non-pathological forms of anxiety (e.g., stage fright or exam nerves) or may be useful as dietary augmentation to conventional pharmacotherapy of anxiety and depression. Whether binding of ginger constituents to the intestinal 5-HT_{1A}R can be expected to ease emesis and nausea in humans is open to question. However, there is strong evidence from different animals (e.g., dogs, cats, pigeons and house musk shrew) that targeting the 5-HT_{1A}R subtype is at least as effective as 5-HT_3 receptor antagonism for the treatment of vomiting or motion sickness.[30] Ginger may therefore find its way into the fast growing market of pet health care. However, further studies are necessary to examine the 5-HT_{1A}R-dependent effects in vivo.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.02.062.

References and notes

1. Goodwin, G. M.; De Souza, R. J.; Green, A. R.; Heal, D. J. *Psychopharmacology* **1987**, *91*, 506.
2. Bjorvatn, B.; Ursin, R. *Rev. Neurosci.* **1998**, *9*, 265.
3. Hutson, P. H.; Dourish, C. T.; Curzon, G. *Eur. J. Pharmacol.* **1988**, *150*, 361.
4. Miczek, K. A.; Hussain, S.; Faccidomo, S. *Psychopharmacology* **1998**, *139*, 160.
5. Parks, C. L.; Robinson, P. S.; Shenk, T.; Toth, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10734.
6. Mössner, R.; Lesch, K.-P. *Brain Behav. Immun.* **1998**, *12*, 249.
7. Artigas, F.; Celada, P.; Laurelle, M.; Adell, A. *Trends Pharmacol. Sci.* **2001**, *22*, 224.
8. Taylor, D. P.; Moon, S. L. *Neuropeptides* **1991**, 19.
9. Appelberg, B. G.; Syvälahti, E. K.; Koskinen, T. E.; Mehtonen, O. P.; Muhonen, T. T.; Naukkarinen, H. H. *J. Clin. Psychiat.* **2001**, *62*, 448.
10. Newman-Tancredi, A.; Conte, C.; Chaput, C.; Verrièle, L.; Audinot-Bouchez, V.; Lochon, S.; Lavielle, G.; Millan, M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *355*, 682.
11. Ozgoli, G.; Goli, M.; Simbar, M. *J. Altern. Complement. Med.* **2009**, *15*, 243.

12. Mustafa, T.; Srivastava, K. C. *J. Ethnopharmacol.* **1990**, *29*, 267.
13. Srivastava, K. C.; Mustafa, T. *Med. Hypotheses* **1992**, *39*, 342.
14. Lantz, R. C.; Chen, G. J.; Sarihan, M.; Sólyom, A. M.; Jolad, S. D.; Timmermann, B. N. *Phytomedicine* **2006**, *14*, 123.
15. Vishwakarma, S. L.; Pal, S. C.; Kasture, V. S.; Kasture, S. B. *Phytother. Res.* **2002**, *16*, 621.
16. Topic, B.; Hasenohrl, R. U.; Hacker, R.; Huston, J. P. *Phytother. Res.* **2002**, *16*, 312.
17. Huang, Q.; Matsuda, H.; Sakai, K.; Yamahara, J.; Tamai, Y. *Yakugaku Zasshi* **1990**, *110*, 936.
18. Kaur, G.; Kulkarni, S. K. *Eur. J. Nutr.* **2001**, *40*, 127.
19. Abdel-Aziz, H.; Nahrstedt, A.; Petereit, F.; Windeck, T.; Ploch, M.; Verspohl, E. *J. Planta Med.* **2005**, *71*, 609.
20. Abdel-Aziz, H.; Windeck, T.; Ploch, M.; Verspohl, E. J. *Eur. J. Pharmacol.* **2006**, *13*, 136.
21. Wang, W.; Li, C. Y.; Wen, X. D.; Li, P.; Qi, L. W. *J. Chromatogr., B Anal. Technol. Biomed. Life Sci.* **2009**, *877*, 671.
22. Jiang, S. Z.; Wang, N. S.; Mi, S. Q. *Biopharm. Drug Dispos.* **2008**, *29*, 529.
23. Ding, G. H.; Naora, K.; Hayashibara, M.; Katagiri, Y.; Kano, Y.; Iwamoto, K. *Chem. Pharm. Bull. (Tokyo)* **1991**, *39*, 1612.
24. Raymond, J. R.; Fargin, A.; Middleton, J. P.; Graff, J. M.; McNeill Haupt, D.; Caron, M. G.; Lefkowitz, R. J.; Dennis, V. W. *J. Biol. Chem.* **1989**, *36*, 21943.
25. Rothen-Rutishauser, B.; Braun, A.; Günthert, M.; Wunderli-Allenspach, H. *Pharm. Res.* **2000**, *17*, 460.
26. Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J. Z.; Xie, X. Q.; Altmann, K. H.; Karsak, M.; Zimmer, A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9099.
27. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
28. Iwasaki, Y.; Morita, A.; Iwasawa, T.; Kobata, K.; Sekiwa, Y.; Morimitsu, Y.; Kubota, K.; Watanabe, T. *Nutr. Neurosci.* **2006**, *9*, 169.
29. Andrews, G.; Hall, W. *The Mental Health of Australians*; Canberra: Mental Health Branch, Australian Government Department of Health and Aged Care: Australia, 1999.
30. Javid, F. A.; Naylor, R. J. *Pharmacol. Biochem. Behav.* **2006**, *85*, 820.