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Title

2-Arachidonoyl glycerol suppresses gastric emptying via the cannabinoid receptor 1-cholecystokinin signaling pathway in mice

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Abbreviated title

2-Arachidonoyl glycerol suppresses gastric emptying via CB1 and CCK in mice

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Keywords

2-arachidonoyl glycerol, cannabinoid receptor 1, cholecystokinin, gastric emptying

Abstract

2-Monoacylglycerol (2-MAG) is one of the digestion products of dietary lipids. We recently demonstrated that a 2-MAG, 2-arachidonoyl glycerol (2-AG) potently stimulated cholecystokinin (CCK) secretion via cannabinoid receptor 1 (CB1) in a murine CCK-producing cell line, STC-1. CCK plays a crucial role in suppressing postprandial gastric emptying. To examine the effect of 2-AG on gastric emptying, we performed acetaminophen and phenol red recovery tests under oral or intraperitoneal administration of 2-AG in mice. Orally administered 2-AG (25 mg/kg) suppressed the gastric emptying rate in mice, as determined by the acetaminophen absorption test and phenol red recovery test. Intraperitoneal administration of a cholecystokinin A receptor antagonist (0.5 mg/kg) attenuated the gastric inhibitory emptying effect. In addition, both oral (10 mg/kg) and intraperitoneal (0.5 mg/kg) administration of a CB1 antagonist counteracted the 2-AG-induced gastric inhibitory effect. Furthermore, intraperitoneal 2-AG (25 mg/kg) suppressed gastric emptying. These results indicate that 2-AG exhibits an inhibitory effect on gastric emptying in mice, possibly mediated by stimulating both CCK secretion via CB1 expressed in CCK-producing cells and acting on CB1 expressed in the peripheral nerves. Our findings provide novel insights into the 2-MAG-sensing mechanism in enteroendocrine cells and the physiological role of 2-MAG.

Abbreviations

2-AG	2-arachidonoyl glycerol
2-MAG	2-monoacylglycerol(s)
2-OG	2-oleoyl glycerol
AEA	anandamide
20:4n-6	arachidonic acid
CB1	cannabinoid receptor 1
CMC	carboxymethyl cellulose
CCK	cholecystokinin
CCK-A	cholecystokinin A
FA	fatty acid(s)
GLP-1	glucagon-like peptide-1
TAG	triacylglycerol(s)

Introduction

Gastric emptying occurs after meal ingestion and primarily affects the subsequent digestion and absorption of nutrients in the intestine. Luminal nutrients such as lipids, proteins/peptides, and carbohydrates in the small intestine (rather than the stomach contents) are critical for delaying gastric emptying via neuroendocrine pathways such as enteroendocrine and vagal (Luttikhold *et al.* 2013; Hellström *et al.* 2006). Delayed gastric emptying facilitates efficient digestion and absorption of nutrients. Accordingly, the rate of gastric emptying markedly affects postprandial glycemia and lipidemia (Westphal *et al.* 2004; Muramatsu *et al.* 2014; Phillips *et al.* 2015), thereby suppressing the rate of gastric emptying and contributing to the attenuation of postprandial hyperglycemia and/or hyperlipidemia.

In the small intestine, dietary triacylglycerols (TAG) are hydrolyzed to fatty acids (FA) and 2-monoacylglycerols (2-MAG). In contrast to FA (Hunt and Knox 1968; McLaughlin *et al.* 1999), although it has been suggested that 2-oleoyl glycerol (2-OG) inhibits gastric emptying via glucagon-like peptide-1 (GLP-1) secretion (Lauffer *et al.* 2009; Hansen *et al.* 2011; Hansen *et al.* 2012), studies on the inhibitory effects of 2-MAG on gastric emptying are limited.

In a previous study (Marzo *et al.* 2008), intraperitoneal administration of anandamide (AEA), one of the endogenous cannabinoids (endocannabinoids) that regulate food intake and energy balance through cannabinoid receptor 1 (CB1) in the brain and the peripheral tissues (Ueda *et al.* 2011; Gendaszewska-Darmach *et al.* 2019; Di Marzo and Matias 2005), suppressed gastric emptying in mice; this inhibitory effect was counteracted by the intraperitoneal injection of a CB1 antagonist. These results suggest that CB1 activation can suppress gastric emptying; however, the site of action

and its relationship with gastrointestinal hormone secretion remain unknown.

We have recently demonstrated that one of 2-MAG and endocannabinoids, 2-arachidonoyl glycerol (2-AG), potently stimulates cholecystokinin (CCK) secretion via CB1 in the murine enteroendocrine cell line, STC-1 (Ochiai *et al.* 2021). CCK, a gastrointestinal hormone produced by enteroendocrine 'I cells' located in the upper small intestine (Dockray 2012; Rehfeld 2000), plays a major role in suppressing gastric emptying (Liddle *et al.* 1986). Furthermore, CB1 is reportedly expressed in mouse duodenal CCK-producing cells (Argueta *et al.* 2019; Sykaras *et al.* 2012). Accordingly, we hypothesized that 2-AG suppresses gastric emptying via CB1 activation and CCK secretion in enteroendocrine cells.

Herein, to examine this hypothesis, we assessed the effects of orally administered 2-AG on the gastric emptying rate in mice using the acetaminophen absorption test and the phenol red recovery method. In addition, we investigated the molecular and signaling mechanisms involved in the 2-AG-induced effects.

Materials and Methods

Animals and diet

Male C57BL/6J mice (6-week-old) were purchased from Japan SLC (Hamamatsu, Japan) and individually housed in a temperature- and humidity-controlled room (22 ± 2°C, 55 ± 5%), maintained on a 12 h light-dark cycle (8:00-20:00 light period). All animals had free access to water and were fed a laboratory chow containing 49.9% carbohydrate, 24.8% protein, and 4.6% fat (CE-2, CLEA Japan Inc., Tokyo). Experiments were performed after an acclimatization period of ≥ 1 week. Mice were fasted overnight the day before the experiment. The study was approved by the Hokkaido University

Animal Committee, and animals were maintained according to the guidelines for the care and use of laboratory animals at Hokkaido University (permission no. 19-0064).

Reagents

Arachidonic acid (20:4n-6) and acetaminophen were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-AG was purchased from Cayman Chemical (Ann Arbor, MI, USA). Devazepide, a cholecystokinin-A (CCK-A) receptor antagonist, was donated by ML Laboratories (Liverpool, UK), and SR141716A, a selective CB1 antagonist, was purchased from Tocris Bioscience (Ellisville, MO, USA). Unless otherwise specified, all other reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

Acetaminophen test

Test agents were suspended in a vehicle composed of saline, containing 1.5% w/v carboxymethyl cellulose (CMC), 1% w/v acetaminophen, and 2% ethanol. Acetaminophen (100 mg/kg body weight) was used as an absorbable marker to assess the gastric emptying rate (Heading *et al.* 1973; Maida *et al.* 2008). Lipid (2-AG or 20:4n-6; 25 mg/kg body weight)-containing test suspensions or the vehicle were orally administered at a dose of 10 mL/kg body weight. Tail vein blood samples were collected before (0 min) and 15, 30, 60, and 120 min after administration. Blood samples were immediately mixed with heparin (final concentration, 50 IU/mL; Nacalai Tesque, Inc., Kyoto, Japan) and placed on ice. Plasma was separated by centrifugation at 2300 × *g* for 10 min at 4°C, then stored at −80°C until analysis. Plasma acetaminophen concentrations were measured using an acetaminophen detection kit (Kanto Chemical Co., Inc., Tokyo,

104 Japan).

105 In a separate experiment, we compared the effects of oral and intraperitoneal 2-
106 AG on gastric emptying. Mice were divided into control, oral 2-AG, and intraperitoneal
107 2-AG groups. Control mice were intraperitoneally administered sterile saline (10 mL/kg)
108 containing 1.5% CMC and 2% ethanol immediately after oral administration of 10 mL/kg
109 acetaminophen (100 mg/kg) dissolved in the same vehicle. The oral 2-AG group was
110 intraperitoneally administered the same sterile saline solution (10 mL/kg; same as the
111 control group) immediately after orally administering 2-AG (25 mg/kg) suspended in the
112 acetaminophen solution. Finally, the intraperitoneal 2-AG group was intraperitoneally
113 administered 2-AG (25 mg/kg) suspended in sterilized saline containing 1.5% CMC and
114 2% ethanol (10 mL/kg) immediately after oral administration of acetaminophen solution.
115 Tail vein blood samples were collected, and plasma acetaminophen concentrations were
116 determined as described above.

117

118 *RT-PCR*

119 RNA was isolated from the mouse jejunum using a Fast Gene^{TM} RNA Premium
120 Kit (NIPPON Genetics, Tokyo, Japan) according to the manufacturer's instructions.
121 cDNA was prepared from 1 μg RNA using ReverTra Ace® qPCR RT Master Mix with
122 gDNA Remover (TOYOBO, Osaka, Japan) and subjected to PCR using primers based on
123 the mouse CB1 mRNA sequence (GenBank accession number NM001355020; forward
124 primer 5'-CCACCTTCCGTACCATCACC-3', reverse primer 5'-
125 AACCAACGGGGAGTTGTCTC-3') and mouse glyceraldehyde-3-phosphate
126 dehydrogenase (GAPDH) mRNA sequence (GenBank accession number NM008084;
127 forward primer 5'-TCACCACCATGGAGAAGGC-3', reverse primer 5'-

GCTAAGCAGTTGGTGGTGCA-3'). PCR conditions were as follows: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 63.3°C for 30 s, and 72°C for 30 s. In addition, PCR products were separated by 1.5% agarose gel electrophoresis and visualized using Midori Green Advance DNA stain (NIPPON Genetics, Tokyo, Japan).

Phenol red test

2-AG (25 mg/kg body weight) or the vehicle (1.5% w/v CMC and 2% ethanol in saline) was orally administered (10 mL/kg body weight). The suspensions contained phenol red (5 mg/kg body weight), as a non-absorbable marker, in addition to acetaminophen (100 mg/kg body weight) to assess the gastric emptying rate (Feldman and Gibaldi 1968; Nishimukai *et al.* 2003). Portal blood was collected into a syringe containing heparin (final concentration 50 IU/mL), aprotinin (final concentration 500 KIU/mL), and dipeptidyl peptidase-IV inhibitor (final concentration 50 μM, Millipore, MA, U.S.A.), 15 min after oral administration from mice under isoflurane anesthesia (MSD K.K., Tokyo, Japan). Plasma was separated and stored as described previously. Immediately after the procedure, mice were euthanized by exsanguination. The stomach was removed after clamping both the distal end of the esophagus and the proximal end of the duodenum. The stomach content was flushed twice with cold saline, and the washout solution was then collected. The debris was removed by centrifugation at 8400 × *g* for 10 min at 4°C. After adding 1 N NaOH to the supernatant (1/10 volume of the supernatant), the concentration of phenol red was spectrophotometrically measured at 560 nm. The gastric emptying rate was calculated as follows:

Gastric emptying rate (%) = [{the amount of phenol red administered (mg) – the amount of phenol red remaining in the stomach (mg)}/the amount of phenol red

administered (mg)] × 100

Statistical analyses

All values are expressed as mean ± SEM. Statistical analyses were performed using JMP Pro version 14.0.0 software (SAS Institute, Inc., Cary, NC, USA). As described in figure legends, significant differences among groups were determined using Student's *t*-test to compare two groups, whereas Dunnett's post hoc test was employed to compare multiple groups. Statistical significance was set at $p < 0.05$.

Results

Oral administration of 2-AG delayed gastric emptying

We first examined the effects of orally administered 2-AG or 20:4n-6 on gastric emptying in fasted mice using an absorbable marker, acetaminophen (Fig. 1). In all groups, plasma acetaminophen concentrations in tail vein blood increased immediately after oral administration of test liquids, gradually decreasing after 15 or 30 min. Compared with the vehicle, oral administration of 20:4n-6 significantly reduced the appearance of acetaminophen in the tail vein blood at 15 min. In addition, oral administration of 2-AG (25 mg/kg) significantly lowered acetaminophen levels after 15 min.

Involvement of CCK-A receptor in the effect of 2-AG

Next, we examined the involvement of the CCK-A receptor in mediating the effects of oral 2-AG following the intraperitoneal administration of devazepide, a CCK-A receptor antagonist. Compared with the control/vehicle treatment, a single oral administration of 2-AG significantly reduced acetaminophen levels at 15 min (similar to

176 the result shown in Fig. 1) and 30 min (Fig. 2a). Conversely, oral 2-AG did not
177 significantly reduce acetaminophen levels following treatment with intraperitoneal
178 devazepide compared with orally administered vehicle (Fig. 2b).
179
180 *Involvement of CB1 in the effect of 2-AG*
181 2-AG is a ligand for CB1, an endocannabinoid receptor (Ueda *et al.* 2011;
182 Gendaszewska-Darmach *et al.* 2019). As determined by conventional PCR, single bands
183 were detected at the expected DNA size (182 bp) for CB1 in the mouse jejunum (Fig. 3a).
184 To elucidate the involvement of CB1, we next performed the acetaminophen test
185 by administering SR141716A, a selective CB1 antagonist. A single oral administration of
186 2-AG significantly reduced acetaminophen concentrations at 15 min compared with the
187 control group (Fig. 3b); however, no significant difference in acetaminophen levels was
188 observed between control and 2-AG groups treated orally with SR141716A (Fig. 3c).
189 Next, we examined the effect of the intraperitoneally administered SR141716A.
190 Treatment with intraperitoneal SR141716A suppressed the oral 2-AG-induced decrease
191 in acetaminophen levels at 15 min (Fig. 3d and e).
192
193 *Measurement of gastric emptying rate by phenol red test*
194 To further investigate the involvement of CB1 in the inhibitory effect of 2-AG
195 on gastric emptying rate, we performed experiments using an unabsorbable marker,
196 phenol red. We observed that the gastric emptying rate was significantly lower in the oral
197 2-AG group than in the control/vehicle group (Fig. 4a), thus further supporting the gastric
198 inhibitory effect of 2-AG. And no significant differences between the control and 2-AG
199 groups were observed following intraperitoneal treatment with SR141716A (Fig. 4b).

Similar to the phenol red test result, plasma acetaminophen levels in the portal vein were significantly lower in the oral 2-AG group than in the control/vehicle group (Fig. 4c). In contrast, plasma acetaminophen levels did not significantly differ between control and 2-AG groups following intraperitoneal administration of SR141716A (Fig. 4d).

Effect of intraperitoneal 2-AG

Next, we examined the effect of intraperitoneal 2-AG (Fig. 5), given that intraperitoneal administration of the CB1 antagonist attenuated 2-AG-induced effects (Fig. 3 and 4). At an identical dose (25 mg/kg), both oral and intraperitoneal administration of 2-AG significantly attenuated the elevation of plasma acetaminophen concentrations at 15 min. Notably, intraperitoneal administration induced a considerably greater and sustained (~30 min) reduction than oral administration. At 120 min, the acetaminophen concentration in the intraperitoneal 2-AG group was significantly higher than the control group.

Discussion

Notably, FA suppress gastric emptying in a carbon chain length-dependent manner by stimulating the secretion of gastrointestinal hormones such as CCK (Hunt and Knox 1968; McLaughlin *et al.* 1999); however, the effect of 2-MAG on gastric emptying remains to be clarified. Accordingly, the objective of the present study was to examine the effect of 2-AG, which is composed of 20:4n-6 and glycerol, on gastric emptying in mice and to elucidate the mechanism underlying its effect. We observed that oral administration of 2-AG (25 mg/kg) suppressed gastric emptying in mice. Furthermore, the involvement of CCK and cannabinoid receptor CB1 was determined using antagonists

224 against CCK-A and CB1 receptors. These results provide novel insights into the 2-MAG-
225 sensing mechanism in enteroendocrine cells and afford a better understanding of the
226 physiological role of 2-MAG.

227 Oral administration of 2-AG (25 mg/kg) significantly reduced acetaminophen
228 levels at 15 min, similar to 20:4n-6 (25 mg/kg; Fig. 1); however, the time at which the
229 acetaminophen concentration peaked tended to differ between 2-AG (15 min) and 20:4n-
230 6 (30 min). In our previous recent study using the murine enteroendocrine cell line, STC-
231 1 (Ochiai *et al.* 2021), 2-AG was shown to stimulate CCK secretion via CB1, whereas
232 20:4n-6 stimulated CCK secretion via G protein-coupled receptor 120 (GRP120).
233 Although further studies are warranted to elucidate the mechanism underlying the effect
234 of 20:4n-6 *in vivo*, the present results (Fig. 1) suggest that 20:4n-6-rich TAG could
235 effectively exert inhibitory effects on gastric emptying.

236 The dose of 25 mg/kg 2-AG used in the present study was not supraphysiological,
237 as described below. According to previous reports (Hansen *et al.* 2012; Hansen and Vana
238 2019), westerners consume approximately 100 g of lipids (TAG) per day, equivalent to
239 33 g of lipids per meal on the consumption of three meals per day. Therefore, ingesting
240 33 g of lipid in a single meal could provide a maximum of 11 g of 2-MAG in the small
241 intestine. Accordingly, 11 g/55 kg body weight equals 200 mg/kg body weight, which is
242 considerably higher than the dose (25 mg/kg body weight) used in the present study.
243 Furthermore, a previous study has demonstrated enhanced GLP-1 secretion in humans
244 weighing 73-97 kg by administering 2 g of 2-OG into the jejunum (Hansen *et al.* 2011),
245 thus supporting the notion that the dose employed in the present study, i.e., 25 mg/kg (2
246 g/80 kg body weight), is within the physiological range.

247 CCK is mainly recognized by CCK-A receptors expressed on peripheral nerves

and inhibits gastric emptying (Herranz 2003). Treatment with a CCK-A receptor antagonist, devazepide, reversed the gastric inhibitory effect of oral 2-AG (Fig. 2), suggesting the involvement of CCK signaling. Our previous finding (Ochiai *et al.* 2021) that 2-AG promoted CCK secretion in the CCK-producing cell line, STC-1 supports that orally given 2-AG acts on CCK-producing enteroendocrine cells, namely, I cells, to promote CCK secretion.

2-AG is an endocannabinoid, and previous reports have suggested that the endocannabinoid system regulates food intake and energy balance through CB1 in the brain and peripheral nerves (Di Marzo and Matias 2005; Sharkey and Pittman 2005; Osei-Hyiaman *et al.* 2006). In the present study, we detected that CB1 was expressed in the jejunum of mice (Fig. 3a). This result is consistent with previous findings that revealed CB1 expression throughout the mouse gastrointestinal tract (Casu *et al.* 2003). CB1 expressed in the intestinal tract is likely involved in mediating the effect of oral 2-AG on gastric emptying, as oral treatment with a CB1 antagonist, SR141716A, counteracted the effect of oral 2-AG (Fig. 3b and c). Furthermore, CB1 expression has been confirmed in murine CCK-producing cells (Argueta *et al.* 2019; Sykaras *et al.* 2012). We recently demonstrated that 2-AG potently stimulates CCK secretion via CB1 expressed in the CCK-producing cell line, STC-1 (Ochiai *et al.* 2021). Therefore, 2-AG might stimulate CCK secretion by acting on CB1 expressed in CCK-producing enteroendocrine cells, thereby suppressing gastric emptying.

Intraperitoneal CB1 antagonists also reversed the gastric inhibitory effect of oral 2-AG in the acetaminophen and phenol red tests (Fig. 3d, e, and 4). These findings suggest that after absorption by intestinal epithelial cells, 2-AG suppresses gastric emptying by acting on CB1 expressed on the basolateral side of CCK-producing cells or in peripheral

nerves. Consistent with this notion, intraperitoneal 2-AG potently suppressed gastric emptying compared with oral 2-AG (Fig. 5). Furthermore, in a previous study (Marzo *et al.* 2008), intraperitoneal AEA-suppressed gastric emptying was reversed following intraperitoneal administration of a CB1 antagonist.

Treatment with a CCK-A receptor or CB1 antagonist did not completely counteract the 2-AG-induced gastric emptying inhibition (Fig. 2, 3, and 4). These results may be attributed to the insufficient inhibition of CCK-A or CB1 under experimental conditions (although the doses used in the present study were selected based on previous reports (Chen *et al.* 2012; Hira *et al.* 2015; Madsen *et al.* 2009; Orio *et al.* 2011; Marzo *et al.* 2008)) or the involvement of additional mechanisms. A previous study (Burdyga 2004) has shown that vagal afferent neurons expressing CCK-A receptors also express CB1; the expression of CB1 in the nodose ganglia is increased by fasting and inhibited by CCK. Additionally, the CCK-A receptor antagonist, lorglumide, blocks the loss of CB1 expression in afferent neurons after refeeding (Burdyga 2004). Based on the results of these previous reports and those noted in the present study, the following pathways are potential mechanisms of action of 2-AG: When CCK-A receptors are inhibited, CB1 activation by 2-AG in peripheral nerves contributes to the suppression of gastric emptying. On inhibiting CB1 on the peripheral nerves and the basolateral side of intestinal epithelial cells, CCK release by 2-AG via activation of CB1 expressed on the apical side of I cells contributes to suppressing gastric emptying. Treatment with a combination of the CCK-A receptor antagonist and an oral or intraperitoneal CB1 antagonist could clarify these possibilities in future studies.

Compared with oral administration (Fig. 5), intraperitoneal administration of 2-AG, at the same dose (25 mg/kg), suppressed gastric emptying more potently and

296 continuously (~30 min). As the result of potent inhibition of gastric emptying, the plasma
297 acetaminophen concentration peaked much later (at 60 min) than other groups (peaked at
298 15 min). Accordingly, plasma acetaminophen concentration at 120 min was maintained
299 higher in the intraperitoneal 2-AG group than the other two groups. There are two possible
300 reasons for the more potent effect of intraperitoneal administration of 2-AG than oral 2-
301 AG. First, only a certain portion of orally administered liquid flows into the small
302 intestine over a short period, and the stomach and intestinal fluids dilute the liquid.
303 Second, only a fraction of 2-AG is transferred to the extracellular side of the basolateral
304 membrane, as 2-AG is degraded by hydrolytic enzymes such as monoacylglycerol lipase
305 expressed in the cell membrane and cytoplasm of intestinal epithelial cells (Dinh *et al.*
306 2002a; Dinh *et al.* 2002b; Blankman *et al.* 2007) and resynthesized into TAG
307 intracellularly (Mu and Høy 2004). Further studies are needed to confirm the presence of
308 2-AG in the lumen after oral administration and the extracellular transfer after absorption.
309 As rapid gastric emptying contributes to postprandial hyperglycemia and
310 lipidemia, the suppression of gastric emptying is a promising target for preventing or
311 reducing glucose intolerance and dyslipidemia. The limitation of the current study is that
312 the gastric inhibitory effect of 2-AG was not compared with that of other 2-MAG because
313 we primarily focused on *in vivo* effect of 2-AG. Although beyond the scope of our current
314 study, comparing their effects would provide valuable insights into the physiological role
315 of 2-MAG and warrant future investigation. Additionally, further studies are required to
316 verify the effects of dietary lipid-derived 2-MAG, including 2-AG, on postprandial blood
317 glucose levels and these diseases.
318 In conclusion, oral administration of 2-AG suppressed gastric emptying in mice,
319 as assessed by the acetaminophen and phenol red tests. Furthermore, studies using CCK-

A receptor or CB1 antagonists suggested the involvement of CCK and CB1 expressed on peripheral nerves and CCK-producing enteroendocrine cells. These results demonstrate that oral 2-AG suppresses gastric emptying in mice via the CB1-CCK and/or CB1 signaling pathways. Thus, our findings indicate a novel physiological interaction between 2-AG and neuroendocrine systems that regulate the gastric emptying rate.

Author Contributions

K.O. and T.H. conceived and designed the study and wrote the first draft of the manuscript; K.O. performed the experiments; K.O. and T.H. analyzed the data. K.O., R.H., M.S., S.T., and T.H. contributed to and approved the final draft of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

Fig. 1 Effects of oral administration of 2-AG or 20:4n-6 on gastric emptying. 2-AG or 20:4n-6 (25 mg/kg) was orally administered to fasted mice along with acetaminophen. Blood samples were collected from the tail vein for up to 120 min, and plasma acetaminophen concentrations were measured. Values are expressed as changes from the basal (0 min) concentration of plasma acetaminophen (ΔAcetaminophen) and as the mean ± SEM (n = 6). Plots with asterisks (*) show significant differences compared with the control group at the same time point ($^{*}p < 0.05$ and $^{**}p < 0.01$, Dunnett's test). 2-AG, 2-arachidonoyl glycerol; 20:4n-6, arachidonic acid

Fig. 2 Effects of 2-AG on gastric emptying in the absence (a) or presence (b) of the CCK-A receptor antagonist (devazepide). Devazepide (0.5 mg/kg) or vehicle (10% TWEEN80 + 10% DMSO in sterilized saline) was intraperitoneally injected at a dose of 5 mL/kg, 15 min before the oral administration of 2-AG (0 or 25 mg/kg) and acetaminophen (100 mg/kg) in fasted mice. Values are expressed as changes from the basal (0 min) concentration of plasma acetaminophen (ΔAcetaminophen) and as the mean ± SEM (n = 6). Plots with asterisks (*) show significant differences compared with the control group at the same time point ($^{*}p < 0.05$, Student's *t*-test). NS indicates that there was no significant difference between treatments. 2-AG, 2-arachidonoyl glycerol; Dvz, devazepide; CCK, cholecystokinin

Fig. 3 Expression of CB1 in the mouse jejunum (a) and effects of 2-AG on gastric emptying in the absence (b, d) or presence (c, e) of CB1 receptor antagonist (SR141716A). (a) Total RNA was extracted from the mouse jejunum and then subjected to RT-PCR with

specific CB1 or GAPDH primers. PCR products were separated in agarose gel and visualized by Midori Green Advance DNA stain. (b, c) 2-AG (25 mg/kg) was orally administered to fasted mice with SR141716A (10 mg/kg) or vehicle (saline containing 1.5% w/v CMC, 1% w/v acetaminophen, 10% TWEEN80, 10% DMSO, and 2% ethanol). (d, e) SR141716A (0.5 mg/kg) or vehicle (10% TWEEN80 + 10% DMSO in sterile saline) was intraperitoneally injected at a dose of 5 mL/kg, 15 min before the oral administration of 2-AG (0 or 25 mg/kg) and acetaminophen in fasted mice. Values are expressed as changes from the basal (0 min) concentration of plasma acetaminophen (ΔAcetaminophen) and as the mean ± SEM (n = 5-6). Plots with asterisks (*) show significant differences compared with the control group at the same time point ($^{*}p < 0.05$ and $^{**}p < 0.01$, Student's *t*-test). NS indicates that there was no significant difference between the treatments. 2-AG, 2-arachidonoyl glycerol

Fig. 4 Effects of 2-AG on the gastric emptying rate in the absence (a, c) or presence (b, d) of SR141716A (phenol red recovery method). 2-AG (25 mg/kg) was orally administered to fasted mice along with phenol red and acetaminophen, 15 min after the intraperitoneal injection of SR141716A (0.5 mg/kg) or vehicle (10% TWEEN80 + 10% DMSO in sterilized saline). Stomach contents and portal blood were collected 15 min after oral administration. (a, b) The gastric emptying rate was determined by measuring luminal phenol red collected from the stomach. (c, d) Acetaminophen levels in the portal plasma were measured. Values are expressed as the mean ± SEM (n = 5-6). Plots with asterisks (*) show significant differences compared to the control group ($^{*}p < 0.05$ and $^{**}p < 0.01$, Student's *t*-test). NS indicates that there was no significant difference between the treatments. 2-AG, 2-arachidonoyl glycerol

Fig. 5 Effect of intraperitoneal administration of 2-AG on gastric emptying. 2-AG (25 mg/kg) was orally or intraperitoneally coadministered (10 mL/kg) to fasted mice with oral acetaminophen. Values are expressed as changes from the basal (0 min) concentration of plasma acetaminophen (ΔAcetaminophen) and as the mean ± SEM (n = 6). Plots with asterisks (*) show significant differences compared to the control group at the same time point ($^{*}p < 0.05$ and $^{**}p < 0.01$, Dunnett's test). 2-AG, 2-arachidonoyl glycerol

Fig. 1

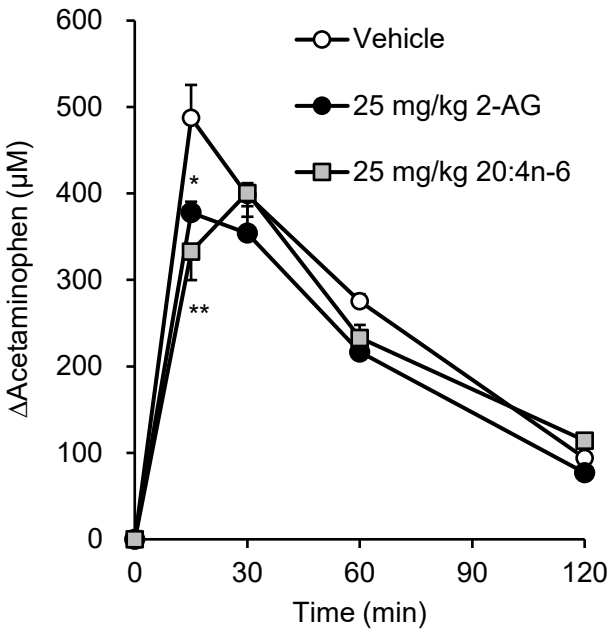

600
500
400
300
200
100
0
ΔAcetaminophen (μM)
0
30
60
90
120
Time (min)
Vehicle
25 mg/kg 2-AG
25 mg/kg 20:4n-6
*
**

Fig. 2

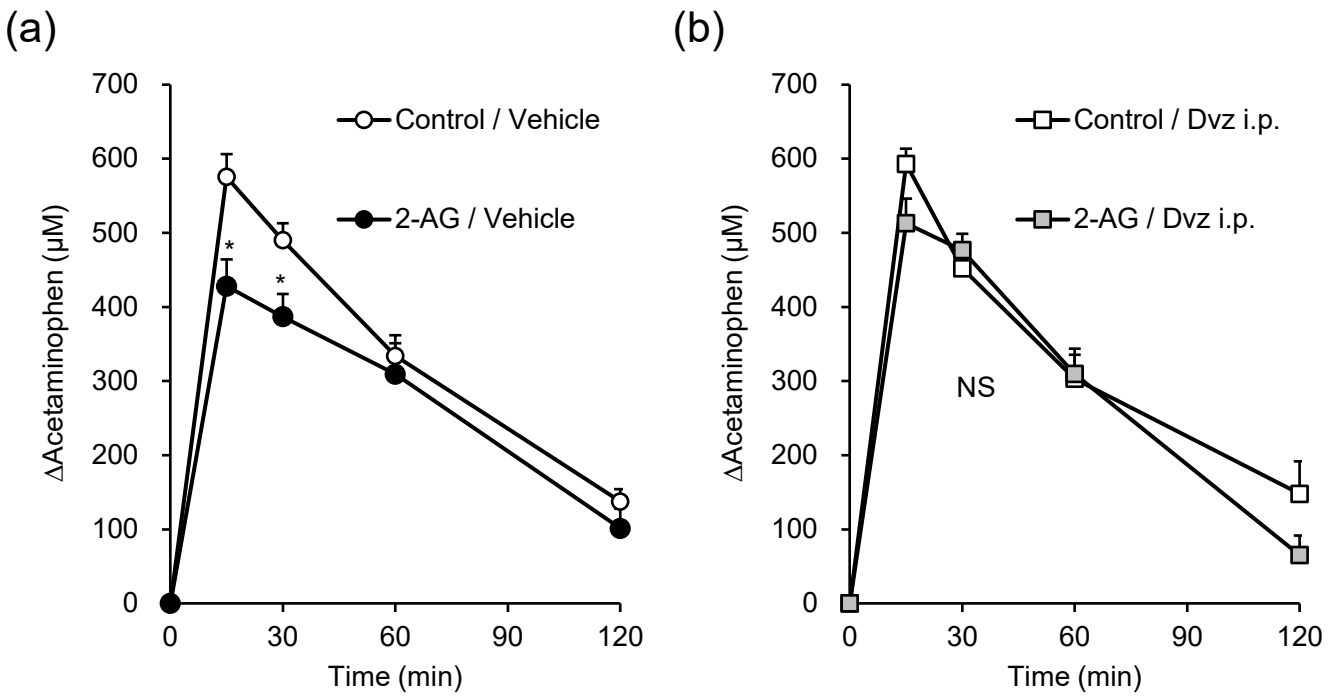

(a)
700
600
500
400
300
200
100
0
ΔAcetaminophen (μM)
Control / Vehicle
2-AG / Vehicle
*
*
0
30
60
90
120
Time (min)
(b)
700
600
500
400
300
200
100
0
ΔAcetaminophen (μM)
Control / Dvz i.p.
2-AG / Dvz i.p.
NS
0
30
60
90
120
Time (min)

Fig. 3

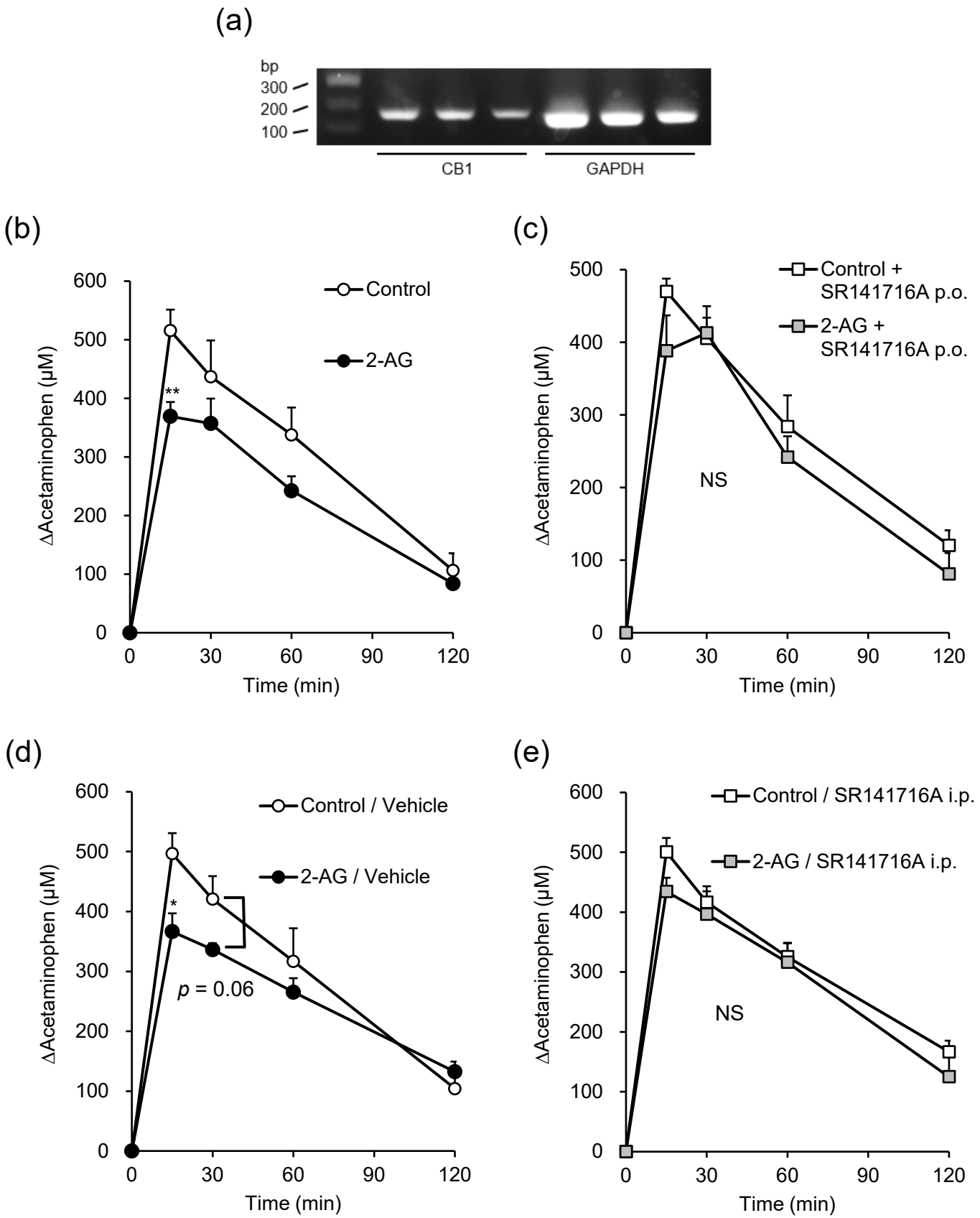

(a)
bp
300
200
100
CB1
GAPDH
(b)
Control
2-AG
**
ΔAcetaminophen (μM)
Time (min)
(c)
Control +
SR141716A p.o.
2-AG +
SR141716A p.o.
NS
ΔAcetaminophen (μM)
Time (min)
(d)
Control / Vehicle
2-AG / Vehicle
*
p = 0.06
ΔAcetaminophen (μM)
Time (min)
(e)
Control / SR141716A i.p.
2-AG / SR141716A i.p.
NS
ΔAcetaminophen (μM)
Time (min)

Fig. 4

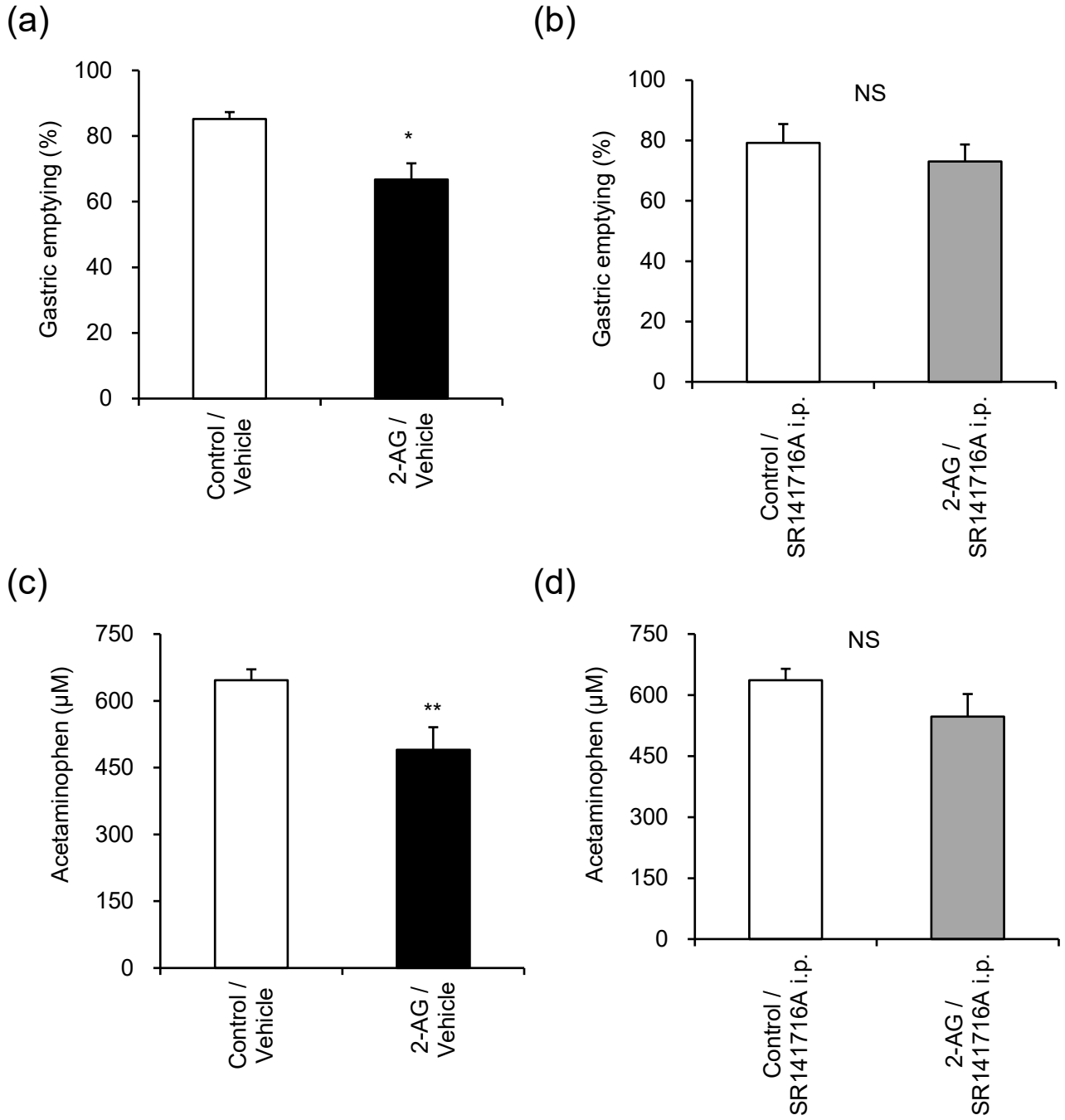
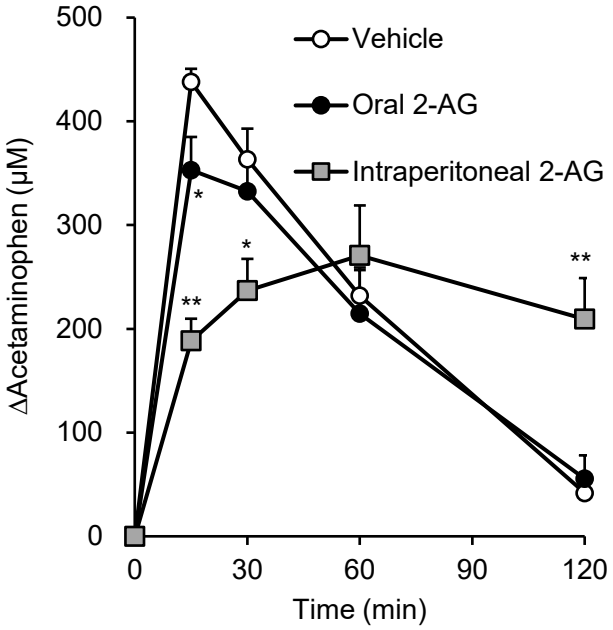

(a)
Gastric emptying (%)
100
80
60
40
20
0
*
Control / Vehicle
2-AG / Vehicle
(b)
Gastric emptying (%)
100
80
60
40
20
0
NS
Control / SR141716A i.p.
2-AG / SR141716A i.p.
(c)
Acetaminophen (μM)
750
600
450
300
150
0
**
Control / Vehicle
2-AG / Vehicle
(d)
Acetaminophen (μM)
750
600
450
300
150
0
NS
Control / SR141716A i.p.
2-AG / SR141716A i.p.

Fig. 5


500
400
300
200
100
0
ΔAcetaminophen (μM)
0
30
60
90
120
Time (min)
Vehicle
Oral 2-AG
Intraperitoneal 2-AG
*
*
**
**

Title

2-Arachidonoyl glycerol suppresses gastric emptying via the cannabinoid receptor 1-cholecystokinin signaling pathway in mice

Journal

Lipids

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