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Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (SAB378), a Peripherally Restricted Cannabinoid CB_1/CB_2 Receptor Agonist, Inhibits Gastrointestinal Motility but Has No Effect on Experimental Colitis in Mice

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ABSTRACT

The endocannabinoid system is involved in the regulation of gastrointestinal (GI) motility and inflammation. Using the peripherally restricted cannabinoid $(CB)_1/CB_2$ receptor agonist naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (SAB378), we investigated the role of peripheral cannabinoid receptors in the regulation of GI motility and the development of colitis in mice. The actions of SAB378 on whole gut transit, upper GI transit, colonic propulsion, and locomotor activity were investigated in C57BL/6N, CB_1 receptor knockout, and CB_2 receptor knockout mice. The potential for SAB378 to modify inflammation was studied by using dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) models of experimental colitis. SAB378 did not modify locomotor activity. SAB378 slowed all parameters of GI motility, and these effects were significantly reduced by the CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3 carboxamide (AM251), but not by the CB_2 receptor antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone (AM630). SAB378 did not inhibit GI transit or colonic propulsion in CB_1 receptor knockout mice, whereas its effects were observed in CB_2 receptor knockout mice. SAB378 did not reduce the degree of colitis induced by DSS or TNBS. The actions of SAB378 on GI motility are mediated by peripherally located CB_1 receptors. SAB378 was not effective against two models of experimental colitis, which may indicate that peripheral cannabinoid receptor stimulation alone may not be sufficient to mediate the anti-inflammatory effects of cannabinoids.

Introduction

Cannabinoids (CBs) are lipid mediators that activate cannabinoid receptors. Cannabinoids can be of exogenous (synthetic and *Cannabis sativa*-derived compounds) or endogenous origin (endocannabinoids). Endocannabinoids, such as 2-arachidonoyl glycerol and anandamide, are produced on demand from the phospholipid precursors present in the cell membrane and bind to CB_1 and/or CB_2 receptors (De Petrocellis and Di Marzo, 2009). Endocannabinoids are degraded by the enzymes fatty acid amide hydrolase and/or monoacylglycerol lipase (De Petrocellis and Di Marzo, 2009). Cannabinoid receptors are known to mediate a number of actions in the gastrointestinal (GI) tract, including the stimulation of feeding and the inhibition of emesis, gastric secretion, gastroesophageal reflux, and transepithelial ion transport (Izzo

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ABBREVIATIONS: CB, cannabinoid; GI, gastrointestinal; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3 carboxamide; AM630, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone; CNS, central nervous system; DSS, dextran sulfate sodium; MPO, myeloperoxidase; SAB378, naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone; TNBS, 2,4,6-trinitrobenzene sulfonic acid; WIN55212-2, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate; HU210, (6a*R*,10a*R*)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol; DMSO, dimethyl sulfoxide; THC, tetrahydrocannabinol.

and Sharkey, 2010). In addition, cannabinoids are known to inhibit GI transit and reduce the degree of intestinal inflammation in animal models of experimental colitis (Izzo and Sharkey, 2010). The development of cannabinoids as therapies for GI motility disorders and inflammatory bowel disease is hampered by their centrally mediated, psychoactive side effects. Although studies have aimed to elucidate whether these cannabinoid effects are mediated through peripherally or centrally located cannabinoid receptors, a definitive answer has not been forthcoming because of a previous lack of pharmacological tools whose actions are restricted to the periphery. The GI tract is under hierarchical neural control with extrinsic autonomic nerves influencing the activity of enteric neurons located within the wall of the gut (Furness, 2006). Because CB_1 receptors are located in the dorsal vagal complex, including the dorsal motor nucleus of the vagus (Van Sickle et al., 2001), and in the myenteric plexus of the enteric nervous system (Kulkarni-Narla and Brown, 2000; Van Sickle et al., 2001; Coutts et al., 2002; Mascolo et al., 2002; Pinto et al., 2002) it follows that CB_1 activation in either of these regions could alter GI motility. Studies have attempted to elucidate, through the use of varying routes of drug administration and/or vagotomy or ganglionic blockade, the role that central versus peripheral CB receptor activation plays in the modulation of GI motility. Their findings revealed evidence that suggests there may be regional differences such that CB_1 receptors located in the vago-vagal circuitry mediate the actions in slowing gastric motility (Krowicki et al., 1999), whereas mainly peripheral CB_1 receptors mediate the inhibitory actions on GI transit (Landi et al., 2002), and either central or peripheral CB_1 receptors can mediate the slowing of upper GI transit and colonic propulsion (Izzo et al., 2000; Pinto et al., 2002).

Experimental colitis is reduced by CB_1 and CB_2 receptor agonists (Kimball et al., 2006; Engel et al., 2008; Storr et al., 2009) and fatty acid amide hydrolase inhibitors (D'Argenio et al., 2006; Storr et al., 2008). There is little evidence to suggest whether cannabinoid-induced attenuation of colitis is mediated by peripheral and/or central receptors. Peripheral levels of CB_1 receptor expression (Izzo et al., 2001; Massa et al., 2004; Kimball et al., 2006), CB_2 receptor mRNA (Storr et al., 2009), and anandamide levels (D'Argenio et al., 2006) are increased in the inflamed intestine, suggesting peripheral receptors may be important in mediating the effects of cannabinoids. It has been shown, in a model of peritonitis, that cannabinoids mediate a protective effect via central CB_1, but not CB_2, receptors (Smith et al., 2001).

A novel CB_1/CB_2 receptor agonist (IC_{50} values of 15 ± 5 and 98 ± 7.6 nM at human CB_1 and human CB_2 receptors, respectively), naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl) methanone (SAB378), with restricted central actions has been described previously (Dziadulewicz et al., 2007). SAB378 is antihyperalgesic and lacks centrally mediated side effects such as hypomotility in rats, and it does not accumulate in the CNS after twice-daily oral doses over 5 days (Dziadulewicz et al., 2007). Furthermore, SAB378 has been shown to have good oral bioavailability in rats (Dziadulewicz et al., 2007), dogs (Trevaskis et al., 2009), and humans (Gardin et al., 2009). We used this compound to study the role of peripheral cannabinoid receptors in the mediation of actions on GI motility and experimental colitis in mice. We used two well established models of experimental colitis, dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS). DSS-induced colitis is characterized by bloody diarrhea, epithelial ulceration, and mucosal neutrophil infiltration, whereas TNBS colitis is characterized by transmural inflammation, ulceration, and neutrophil infiltration.

Using the peripherally restricted cannabinoid agonist SAB378, we report that cannabinoid-induced slowing of GI motility is mediated by peripheral CB_1 receptor activation, whereas the protective actions of cannabinoids in experimental colitis may depend on central actions.

Materials and Methods

Animals

Female C57BL/6N mice (17–25 g) and male CD1 mice (25–37 g) were purchased from Charles River Canada (Montreal, QC, Canada). Two breeding pairs of heterozygous CB_1(+/−C57BL/6N) mice were obtained from Dr. B. Lutz (University Medical Center, Mainz, Germany), and two breeding pairs of heterozygous CB_2(+/−C57BL/6) mice were obtained from Dr. N. Buckley (California State Polytechnical University, Pomona, CA) and bred at the University of Calgary to obtain CB_1(−/−C57BL/6N) and CB_2(−/−C57BL/6N) mice, respectively. Animals used in these studies were backcrossed from both heterozygous and homozygous breeding pairs to C57BL/6N for six generations and were used at the same age [female, CB_1(−/−), 8–16 weeks and CB_2(−/−), 6–15 weeks] and maintained under the same conditions as the C57BL/6N and CD1 mice. All CB_1(−/−) (Marsicano et al., 2002) and CB_2(−/−) (Buckley et al., 2000) mice were genotyped by using established protocols and confirmed as homozygous gene-deficient animals [CB_1(−/−C57BL/6N), CB_2(−/−C57BL/6N)] before inclusion in the study. All mice were housed in plastic cages with sawdust floors and allowed free access to tap water and standard laboratory chow, unless otherwise stated. All experimental procedures were approved by the University of Calgary Animal Care Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Drugs

SAB378 was synthesized and supplied by Novartis Pharmaceuticals (Basel, Switzerland). The CB_1 receptor antagonist/inverse agonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3 carboxamide (AM251), the CB_2 receptor antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone (AM630), and the CB_1/CB_2 receptor agonist (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN55212-2) were purchased from Tocris Bioscience (Ellisville, MI). All drugs were dissolved in a vehicle of 2% DMSO and 1% Tween 80 in physiological saline. Injections were administered intraperitoneally at 4 μl/g body weight. DSS (molecular weight 36,000–50,000) was purchased from MP Biomedicals (Solon, OH). TNBS was purchased from Fluka (Buchs, Switzerland).

Locomotor Activity Studies

Ambulatory locomotor activity was measured with an infrared beam activity monitor (Columbus Instruments, Columbus, OH). Sequential breaking of the invisible infrared beams by movement of the mouse was recorded, by the monitor, as the ambulatory activity count. C57BL/6N mice were individually placed in the apparatus, and the ambulatory count was recorded over 10 min. The activity apparatus was cleaned with Virkon spray between subjects. Mice underwent a locomotor activity trial approximately 4 h before the test. All experiments were started at 9:00 AM. For the test, mice were injected intraperitoneally with either vehicle or SAB378 (0.1 or 1.0 mg/kg), and 20 min later they were placed in the activity monitor where ambulatory activity was recorded for 10 min.

In Vivo Transit Studies

Whole Gut Transit Studies. Three days before the experiment, mice were individually housed. On the day of the experiment, mice were transferred to individual plastic cages without bedding and left to acclimatize to the cage for 1 h. C57BL/6N mice were administered an intraperitoneal injection of vehicle, AM251 (1.0 mg/kg), or AM630 (1.0 mg/kg) 20 min before receiving an intraperitoneal injection of vehicle, WIN55212-2 (1.0 mg/kg), or SAB378 (0.1 or 1.0 mg/kg). Twenty minutes later, mice were gavaged (using a 3-cm, 20-G gavaging needle) with 200 μl of an Evans blue marker (5% Evans blue, 5% gum arabic). Mice were returned to their individual cages (ad libitum access to food and water) and the latency to the detection of Evans blue in the droppings was recorded. In additional experiments, CB_1 receptor knockout or CB_2 receptor knockout mice were injected intraperitoneally with vehicle or SAB378 (0.1 or 1.0 mg/kg), and whole gut transit was measured as outlined above.

Upper GI Transit Studies. Mice were fasted for 10 to 14 h before the start of the experiment with ad libitum access to water. C57BL/6N mice were administered an intraperitoneal injection of vehicle, AM251 (1.0 mg/kg), or AM630 (1.0 mg/kg) 20 min before receiving an intraperitoneal injection of vehicle or SAB378 (0.1 or 1.0 mg/kg). Twenty minutes later, mice were gavaged (using a 3-cm, 20-G gavaging needle) with 200 μl of an Evans blue marker (5% Evans blue, 5% gum arabic). Fifteen minutes later, mice were killed via cervical dislocation, and the intestine from the region of the pyloric sphincter to the ileo-caecal junction was removed. Without stretching the tissue, the length of the intestine and distance traveled by the marker was recorded. In other experiments CB_1 receptor knockout mice were injected intraperitoneally with vehicle or SAB378 (1.0 mg/kg), CB_2 receptor knockout mice were injected intraperitoneally with vehicle or SAB378 (0.1 or 1.0 mg/kg), and upper GI transit was measured as outlined above.

Colonic Propulsion. Mice were lightly anesthetized with isoflurane before a 2.5-mm spherical glass bead was inserted 2 cm intrarectally. The latency to the expulsion of the bead was recorded. C57BL/6N mice were injected intraperitoneally with vehicle, AM251 (1.0 mg/kg), or AM630 (1.0 mg/kg) 20 min before receiving an intraperitoneal injection of vehicle, WIN55212-2 (1.0 mg/kg), or SAB378 (0.1 or 1.0 mg/kg). Twenty minutes later, colonic propulsion was recorded. In additional experiments, CB_1 receptor knockout or CB_2 receptor knockout mice were injected intraperitoneally with vehicle or SAB378 (0.1 or 1.0 mg/kg), and colonic propulsion was measured as outlined above.

Experimental Colitis Studies

DSS-Induced Colitis. Male CD1 mice were administered DSS (5% DSS in drinking water, days 0–5), and body weight was recorded daily. On days 4 to 8 post-DSS initiation vehicle, WIN55212-2 (2.0 mg/kg), or SAB378 (0.1 and 1.0 mg/kg) was administered intraperitoneally twice daily (9:00 AM and 5:00 PM). On day 8 mice were killed via cervical dislocation. The colon was dissected and assessed for macroscopic evidence of colitis by a blinded investigator. Body weight score was calculated as the percentage weight loss from the initial body weight on day 0 (1 = 0–5%, 2 = 5.1–10%, 3 = 10.1–15%, 4 > 15%). Colon length score was calculated as a percentage of control colon length (1 = 75–85%, 2 = 65–74.9%, and 3 < 64.9%). The presence (score = 1) or absence (score = 0) of erythema, fecal blood, and diarrhea was recorded. A total macroscopic damage score was calculated for each animal comprising body weight score, colon length score, erythema score, fecal blood score, diarrhea score, and length of inflamed colon as percentage of total length. Myeloperoxidase (MPO) activity was measured to assess neutrophil infiltration as described previously (Storr et al., 2009).

TNBS-Induced Colitis. Male CD1 mice were intraperitoneally injected with SAB378 (0.1 and 1.0 mg/kg), WIN55212-2 (2 mg/kg), or vehicle. One hour later, mice were lightly anesthetized with isoflurane before the intrarectal administration of TNBS (100 μl of 40 mg/ml in 30% ethanol). Mice were injected with SAB378 (0.1 and 1.0 mg/kg), WIN55212-2 (2 mg/kg), or vehicle 8 and 24 h after the initial drug injection. Three days post-TNBS application mice were killed via cervical dislocation. The colon was dissected and assessed for macroscopic evidence of colitis by a blinded investigator. The length of the colon was measured and scored accordingly (>8.1 cm = 0, 7.1–8 cm = 1, and <7 cm = 2). The presence (score = 1) or absence (score = 0) of erythema, fecal blood, and diarrhea was recorded. A total macroscopic damage score was calculated for each animal comprising colon length score, length of inflamed colon (percentage total colon length), erythema score, fecal blood score, diarrhea score, and length of ulcerated colon (cm). MPO activity was measured as noted above. Tissue was also collected for microscopic analysis. Tissues were first fixed overnight in Zamboni's fixative at 4°C then washed three times at 10-min intervals in phosphate-buffered saline. Tissues were cryo-protected in phosphate-buffered saline–sucrose (20%) then embedded in optimal cutting temperature compound. Sections were cut (14 μm) using a cryostat and stained with hematoxylin and eosin. A microscopic damage score of sections was determined, by two blinded investigators, based on the presence (score = 1) or absence (score = 0) of goblet cell depletion, the presence (score = 1) or absence (score = 0) of crypt abscesses, the destruction of normal architecture (normal = 1, moderate = 2, extensive = 3), the extent of muscle thickening (normal = 1, moderate = 2, extensive = 3), and the presence and degree of cellular infiltration (normal = 1, moderate = 2, transmural = 3).

Statistical Analysis

Data are expressed as the mean ± S.E.M. and were analyzed by using an unpaired *t* test or one-way or two-way analysis of variance (with time as the repeated measure) followed by Bonferroni's post hoc test as appropriate. $P < 0.05$ was considered significant.

Results

Locomotor Activity Studies. The action of SAB378 on locomotor activity in mice was examined to confirm that it did not have actions typical of centrally acting cannabinoid agonists (Dziadulewicz et al., 2007), which would be to reduce locomotor activity (Herkenham, 1992). SAB378 at doses of 0.1 and 1.0 mg/kg had no effect on ambulatory motor activity in mice (Fig. 1), with the number of beam breaks in SAB378-treated mice being comparable with that seen in the vehicle-treated controls.

Whole Gut Transit Assay. Experiments were carried out to examine the action of SAB378 on whole gut transit. The centrally active CB_1/CB_2 receptor agonist WIN55212-2 was used as a reference compound to show established effects of CB_1 receptor activation on transit. WIN55212-2 delayed

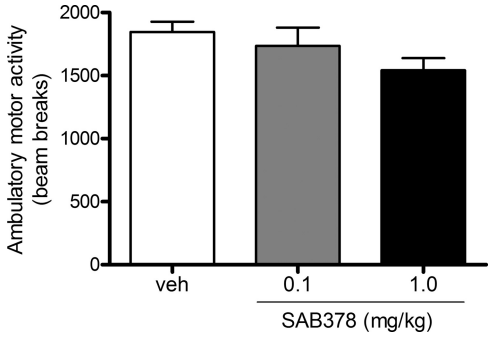

Fig. 1. The effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline) or SAB378 (0.1 or 1.0 mg/kg) on ambulatory locomotor activity, recorded for 10 min, in C57BL/6N mice. All injections were administered intraperitoneally. Bars represent the mean ± S.E.M. (n = 8–11).

whole gut transit, and this effect was blocked by the CB_1 receptor antagonist AM251, but not by the CB_2 receptor antagonist AM630 (Fig. 2A). SAB378 slowed whole gut transit (Fig. 2A). In mice treated with AM630 before SAB378 the inhibitory effect was still observed; however, AM251 significantly reduced the action of SAB378 on transit (Fig. 2A).

To further confirm that the action of SAB378 was mediated through CB_1 receptors, whole gut transit assays were carried out in CB_1 receptor knockout and CB_2 receptor knockout mice. Although 0.1 mg/kg SAB378 slowed whole gut transit to some degree in CB_1 knockout mice (transit was 49.9 ± 12.0% slower than in vehicle-treated CB_1 knockout mice), it was not to the same magnitude as in C57BL/6N mice (transit was 106.9 ± 8.0% slower than in vehicle-treated C57BL/6N mice). SAB378 (1.0 mg/kg) has no effect on whole gut transit in CB_1 knockout mice (Fig. 2B). SAB378 had a significant effect on whole gut transit in CB_2 receptor knockout mice (Fig. 2C). It is noteworthy that the inhibitory effect of SAB378 (1.0 mg/kg) on whole gut transit was much greater in C57BL/6N mice than in CB_2 receptor knockout mice. Whole gut transit in C57BL/6N mice was 174.7 ± 11.9% slower than in vehicle-treated mice, whereas in CB_2 receptor knockout mice transit was only 64.7 ± 3.4% slower than in vehicle-treated mice.

Upper GI Transit Assay. To determine whether a specific region of the GI tract was being inhibited by SAB378, contributing to an overall slowing of whole gut transit, its action on upper GI transit was investigated. SAB378 (1.0 mg/kg) slowed transit of the upper GI tract in a manner that was significantly decreased by AM251 but not AM630 (Fig. 3A). In CB_1 receptor knockout mice upper GI transit in mice treated with SAB378 (1.0 mg/kg) was comparable with that observed in vehicle-treated controls (Fig. 3B). SAB378 inhibited upper GI transit in CB_2 knockout mice (Fig. 3C).

Colonic Propulsion Assay. To further characterize the action of SAB378 on GI transit, its effect on colonic propulsion was examined. The positive control WIN55212-2 slowed colonic propulsion (Fig. 4A). This effect was blocked by AM251 but not AM630 (Fig. 4A). SAB378 (1.0 mg/kg) inhibited colonic propulsion in a manner that was not blocked by AM630 and was significantly reduced by AM251 (Fig. 4A). In CB_1 receptor knockout mice SAB378 did not modify colonic propulsion (Fig. 4B). The lower dose of 0.1 mg/kg SAB378 had no effect on propulsion in CB_2 receptor knockout mice, whereas SAB378 at a dose of 1.0 mg/kg slowed colonic pro-

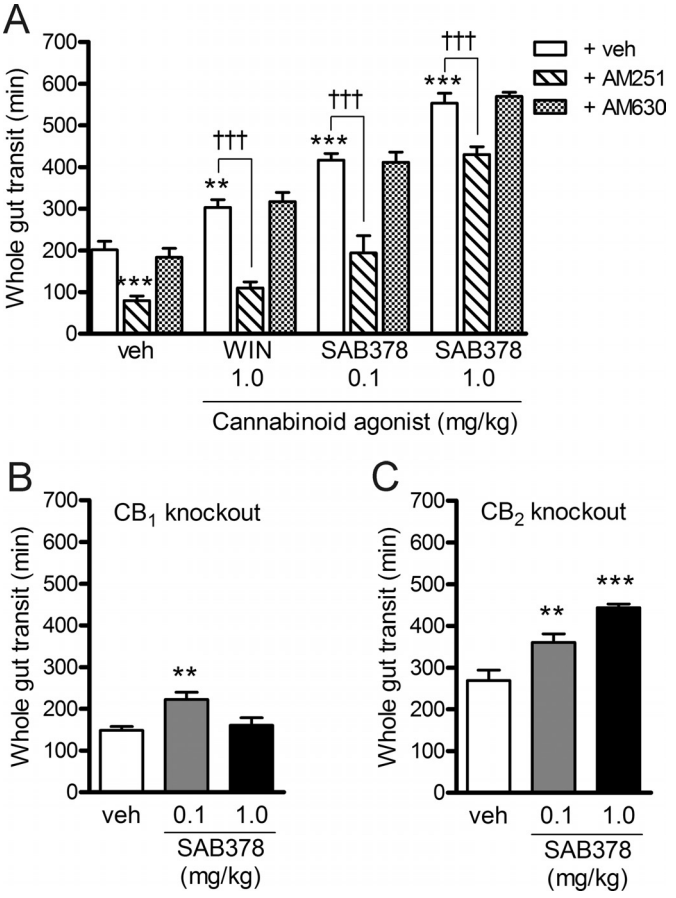

Fig. 2. A, the effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), 1.0 mg/kg AM251, or 1.0 mg/kg AM630 administered 20 min before vehicle, WIN55212-2 (WIN; 1.0 mg/kg), or SAB378 (0.1 or 1.0 mg/kg) on whole gut transit in C57BL/6N mice. B and C, the effect of vehicle or SAB378 (0.1 or 1.0 mg/kg) on whole gut transit in CB_1 (B) and CB_2 (C) knockout mice. All injections were administered intraperitoneally. Bars represent the mean ± S.E.M. (n = 6–11). **, $p < 0.01$ and ***, $p < 0.001$ denote a significant difference to the vehicle-treated control. †††, $p < 0.001$ denotes a significant difference between the indicated groups.

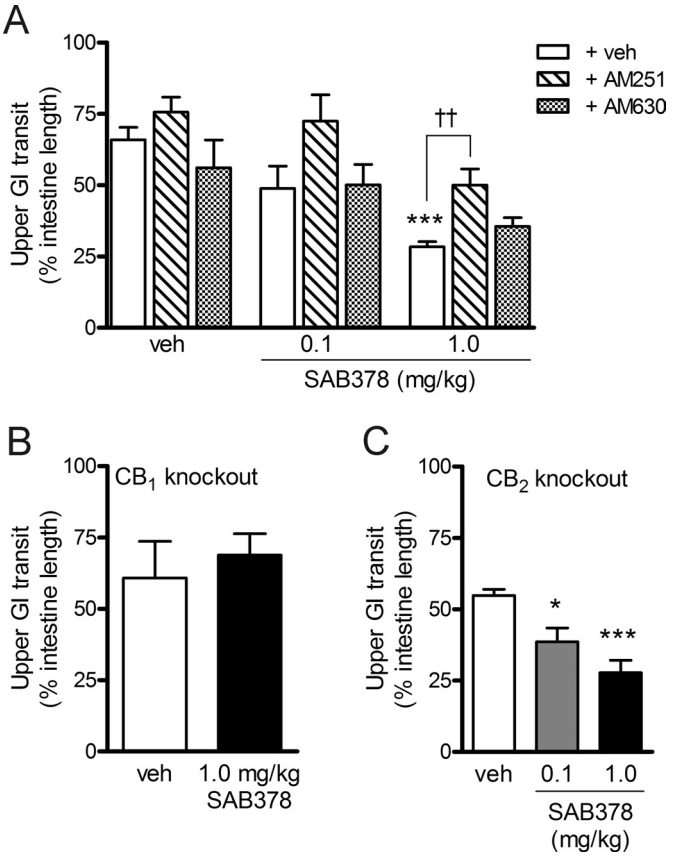

Fig. 3. A, the effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), 1.0 mg/kg AM251, or 1.0 mg/kg AM630 administered 20 min before vehicle or SAB378 (0.1 or 1.0 mg/kg) on upper GI transit in C57BL/6N mice. B and C, the effect of vehicle or SAB378 (1.0 mg/kg) on upper GI transit in CB_1 knockout mice (B) and the effect of vehicle or SAB378 (0.1 and 1.0 mg/kg) on upper GI transit in CB_2 knockout mice (C). All injections were administered intraperitoneally. Bars represent the mean ± S.E.M. (n = 4–8). *, $p < 0.05$ and ***, $p < 0.001$ denote a significant difference to the vehicle-treated control. ††, $p < 0.01$ denotes a significant difference between the indicated groups.

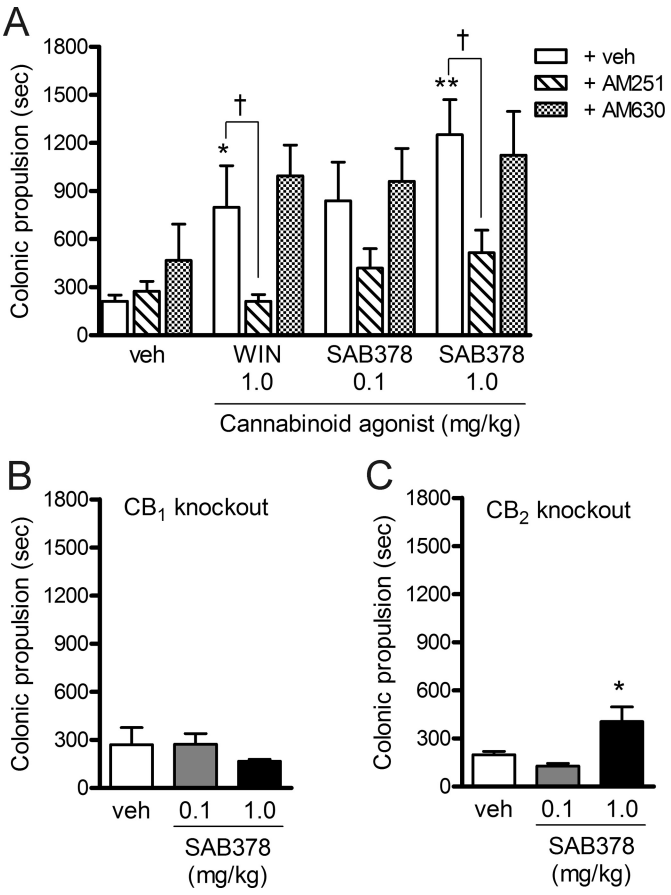

Fig. 4. A, the effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), 1.0 mg/kg AM251, or 1.0 mg/kg AM630 administered 20 min before vehicle, WIN55212-2 (WIN; 1.0 mg/kg), or SAB378 (0.1 or 1.0 mg/kg) on colonic propulsion in C57BL/6N mice. B and C, the effect of vehicle or SAB378 (0.1 or 1.0 mg/kg) on colonic propulsion in CB_1 (B) and CB_2 (C) knockout mice. All injections were administered intraperitoneally. Bars represent the mean ± S.E.M. (n = 6–10). *, $p < 0.05$ and **, $p < 0.01$ denote a significant difference to the vehicle-treated control. †, $p < 0.05$ denotes a significant difference between the indicated groups.

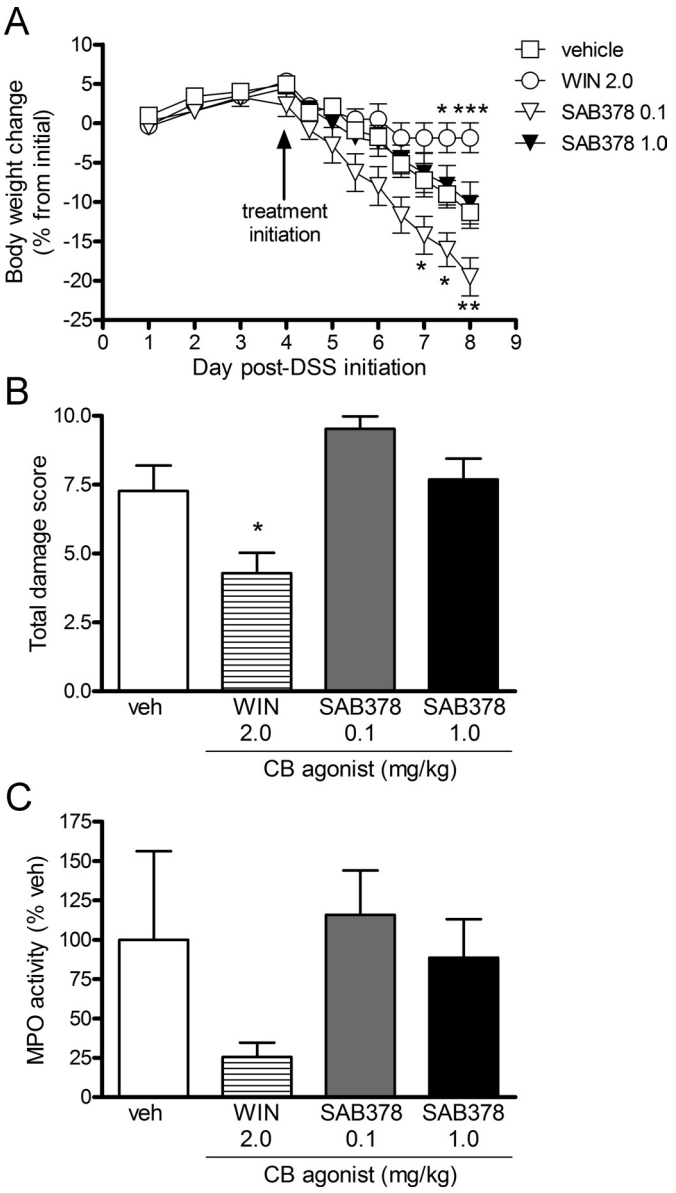

Fig. 5. A, the effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), WIN55212-2 (WIN; 2.0 mg/kg), or SAB378 (0.1 or 1.0 mg/kg), administered twice daily intraperitoneally, on changes in body weight of CD1 mice after DSS administration (5% DSS in drinking water, days 0–5). B and C, mice were killed on day 8, and the effect of vehicle, WIN55212-2, or SAB378 on total macroscopic damage score (B) and MPO activity (C) of the colon was measured. Data points or bars represent the mean ± S.E.M. (n = 5–8). *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$ denote a significant difference to the vehicle-treated control.

pulsion in these mice (Fig. 4C). However, the inhibitory effect of SAB378 (1.0 mg/kg) on colonic propulsion was significantly greater ($p < 0.01$) in C57BL/6N mice than in CB_2 receptor knockout mice. Propulsion in C57BL/6N mice was 491.6 ± 103.9% slower than in vehicle-treated mice, whereas in CB_2 receptor knockout mice transit was only 104.0 ± 45.8% slower than in vehicle-treated mice.

DSS-Induced Colitis. DSS was used to induce colitis in mice. Body weight loss is an indicator that colitis has been established, and vehicle-treated control animals showed an 11.3% loss in body weight from their starting weight on day 0 (Fig. 5A). WIN55212-2 blocked the effect of DSS on body weight change such that by day 6 those mice did not lose further body weight. In fact, DSS mice treated with WIN55212-2 were significantly heavier than their vehicle-treated counterparts on days 7 and 8 (Fig. 5A). SAB378 at the lower dose of 0.1 mg/kg induced a greater body weight loss than the controls, whereas the body weight of mice treated with 1.0 mg/kg SAB378 was comparable with that in the vehicle control mice (Fig. 5A).

The total macroscopic colonic damage score in DSS mice treated with WIN55212-2 was significantly reduced compared with the vehicle controls (Fig. 5B). Although there was a trend for WIN55212-2 to reduce MPO levels in DSS administered mice, indicating a less severe inflammatory response to DSS, it failed to reach significance ($p > 0.05$; Fig. 5C). SAB378 did not modify the macroscopic colonic damage score or MPO activity in DSS mice compared with controls (Fig. 5, B and C).

TNBS-Induced Colitis. To further assess the effect of SAB378 in colitis we carried out investigations in TNBS-induced colitis. In vehicle-treated mice TNBS induced colitis (Fig. 6). WIN55212-2 significantly reduced body weight loss (Fig. 6A) and total macroscopic and microscopic colonic damage score (Fig. 6, B and D) and demonstrated a trend to reduce MPO activity (Fig. 6C) in TNBS-induced colitis.

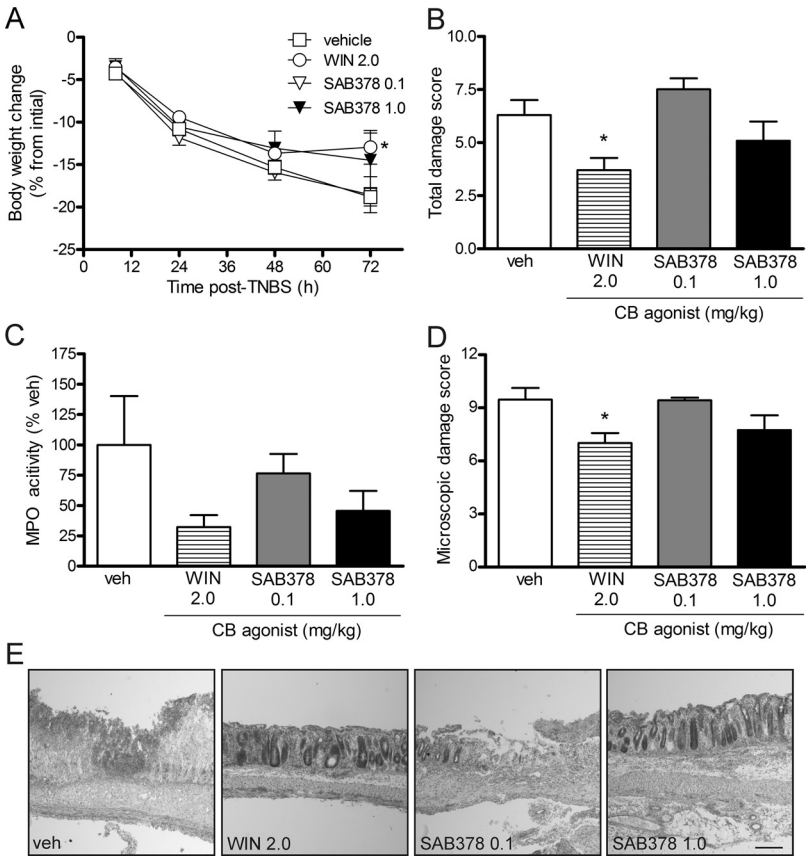

Fig. 6. A, the effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), WIN55212-2 (WIN; 2.0 mg/kg), or SAB378 (0.1 or 1.0 mg/kg), administered intraperitoneally, on changes in body weight of CD1 mice after TNBS administration. B to D, mice were killed on day 3 post-TNBS administration, and the effect of vehicle, WIN55212-2, or SAB378 on total macroscopic damage score (B), MPO activity (C), and microscopic damage score (D) of the colon were measured. Data points or bars represent the mean ± S.E.M. (n = 5–8). *, $p < 0.05$ denotes a significant difference to the vehicle-treated control. E, representative photomicrographs of hematoxylin and eosin staining in TNBS-administered colon after vehicle, WIN55212-2, or SAB378 (0.1 and 1.0 mg/kg) treatment. Scale bar represents 100 μm.

SAB378 (0.1 mg/kg) had no effect on these parameters of TNBS-induced colitis compared with vehicle controls (Fig. 6). SAB378 (1.0 mg/kg) demonstrated a nonsignificant trend to reduce body weight loss (Fig. 6A), macroscopic and microscopic damage scores (Fig. 6, B and D), and MPO activity (Fig. 6D) compared with vehicle-treated controls.

Discussion

We report the actions of a peripherally restricted cannabinoid receptor agonist, SAB378, on gastrointestinal motility and in experimental models of colitis. This mixed CB_1/CB_2 receptor agonist inhibited gastrointestinal transit; however, SAB378 had no effect on the degree of inflammation induced in the DSS or TNBS model of colitis. WIN55212-2, which is a centrally active CB_1/CB_2 receptor agonist (Compton et al., 1992), inhibited both GI motility and attenuated colitis.

It has been shown that orally administered SAB378 does not induce typical cannabinoid-mediated CNS effects, such as catalepsy, at doses and time points that are antihyperalgesic (Dziadulewicz et al., 2007). This, along with pharmacokinetic studies showing a high affinity for plasma protein, thus limiting the passage of SAB378 across the blood-brain barrier, suggest that this compound is peripherally restricted (Dziadulewicz et al., 2007). We confirmed this property in the present study by demonstrating that SAB378, when administered intraperitoneally, does not significantly reduce locomotor activity in mice. The induction of hypomotility by cannabinoid agonists (Little et al., 1988) is believed to be a centrally mediated event caused by the high density of CB_1 receptors in the basal ganglia, and it is likely that activation of these receptors results in impaired locomotor activity (Herkenham, 1992).

It is well established that cannabinoids exert a braking effect on physiological GI transit, inhibiting gastric emptying and motility (Shook and Burks, 1989; Izzo et al., 1999b; Krowicki et al., 1999), upper GI transit (Shook and Burks, 1989; Colombo et al., 1998; Izzo et al., 1999a, 2000, 2001; Landi et al., 2002; Mathison et al., 2004), and colonic propulsion (Pinto et al., 2002), through the activation of CB_1 receptors. In the current study, through the use of specific CB_1 and CB_2 receptor antagonists, we confirmed the CB_1-mediated inhibitory action of WIN55212-2 on GI tract motility and colonic propulsion. Furthermore, we report that SAB378 also slows whole gut transit and upper GI transit in a CB_1-mediated manner. Although the lower dose of SAB378 (0.1 mg/kg) slowed whole gut transit in CB_1 receptor knockout mice this was not to the same degree as was observed in control C57BL/6N mice, and the higher dose (1.0 mg/kg) was without transit effects in these mice. Furthermore, the CB_1 receptor antagonist AM251 completely reversed 0.1 mg/kg SAB378-induced inhibition of whole gut transit. SAB378 did not modify upper GI transit in CB_1 receptor knockout mice, and AM251 reversed SAB378-induced inhibition of upper GI transit in C57BL/6N mice. The CB_2 receptor antagonist AM630 did not modify the SAB378-induced effect on whole gut transit or upper GI transit, and the inhibitory effect in both parameters was observed in CB_2 receptor knockout mice, suggesting that CB_2 receptors are not involved in these actions of SAB378. Under physiological conditions GI motility is not modified by CB_2 receptor agonists (Mathison et al., 2004), and the inhibitory action of mixed CB_1/CB_2 receptor

agonists is not blocked by CB_2 receptor antagonists (Izzo et al., 1999b, 2000; Pinto et al., 2002). Thus it was not surprising to observe in our study that SAB378 was not acting via CB_2 receptors to slow physiological upper GI transit.

In the current study, the inhibitory action of SAB378 on colonic propulsion was significantly reversed by AM251, but not AM630, again suggesting that this action is mediated by CB_1 receptor and not CB_2 receptor. SAB378 did not inhibit colonic propulsion in CB_1 receptor-deficient mice, and although there was an inhibition of colonic propulsion in CB_2 receptor-deficient mice after SAB378 administration, it was not to the same magnitude as observed in control C57BL/6N mice. This suggests that activation of CB_1 is essential for the inhibitory action of SAB378 in colonic propulsion. Activation of the CB_2 receptor may not be necessary for SAB378-induced inhibition of colonic propulsion to be seen (as evidenced by pharmacological blockade of the receptor having no effect on the SAB378 action). However, the presence of CB_2 receptors may be required for the full CB_1-mediated inhibitory effect to be revealed. To the best of our knowledge this is the first report of such an interaction between CB_1 and CB_2 receptors, and it seems that, in the regions of the GI tract examined in these studies, this phenomenon is unique to the colon.

Whole gut transit was also inhibited by SAB378 in a CB_1 receptor-mediated manner, as determined through the use of cannabinoid receptor-specific antagonists and cannabinoid receptor gene-deficient mice. In CB_2 receptor knockout mice, SAB378 did not inhibit whole gut transit to the same degree as observed in the C57BL/6N mice, and this is probably caused by SAB378 having a blunted effect on colonic propulsion in these mice.

Overall, in all of the regions examined, SAB378 inhibited gastrointestinal transit, suggesting that cannabinoid-induced slowing of GI motility is mediated by peripherally located receptors. It has been suggested, via the systemic and/or central administration of cannabinoid agonists and antagonists, that mainly peripheral CB_1 receptors are involved in cannabinoid-induced modulation of gastrointestinal transit (Landi et al., 2002). However, it has also been shown that the CB_1/CB_2 receptor agonist tetrahydrocannabinol (THC) inhibits gastric motility when applied to the dorsal surface of the medulla (Krowicki et al., 1999) and that the inhibitory actions on gastric motility of systemically administered THC were blocked by vagotomy and ganglionic blockade by hexamethonium, suggesting the vago-vagal circuitry is the site of action of THC in this effect (Krowicki et al., 1999). Intracerebroventricular administration of the CB_1/CB_2 receptor agonist WIN55212-2 inhibited upper GI transit in mice with a significantly lower ED_{50} than when injected intraperitoneally, suggesting that slowed transit induced by this agonist is mediated by central CB_1 receptors (Izzo et al., 2000). However, those authors also found that despite ganglionic blockade intraperitoneally injected cannabinoid agonists still slowed upper GI transit, suggesting that peripheral CB_1 receptors mediate the inhibitory effect when systemically administered (Izzo et al., 2000). Likewise, colonic propulsion in mice was slowed after either intracerebroventricular or intraperitoneal administration of the CB_1 receptor agonist arachidonyl-2-chloroethylamide, suggesting that activation of either central or peripheral receptors can induce an effect on the colon (Pinto et al., 2002). We have confirmed that the stimulation of peripheral CB_1 receptors by SAB378 inhibits motility of the whole gut and also upper GI transit and colonic propulsion as separate elements, and peripheral CB_2 receptors may play a role in the mediation of SAB378-induced inhibition of colonic propulsion.

In addition to its actions on GI physiology, the endocannabinoid system is known to exert anti-inflammatory actions in the GI tract. Despite this, it has not been determined whether the effects of cannabinoids on inflammation are mediated by central or peripheral cannabinoid receptors or whether receptors in both regions play a part. As described above, CB_1 receptors are located in the CNS regions primarily involved in GI motor control and in the enteric nervous system, and CB_2 receptors are similarly present in these regions (Van Sickle et al., 2005; Duncan et al., 2008). In experimental colitis a local response to the chemical insult (DSS and TNBS in these studies) is initiated, resulting in the release of proinflammatory cytokines and mediators. Anti-inflammatory responses are also initiated to control the inflammatory response. The balance between the proinflammatory and anti-inflammatory responses is, in part, controlled by the CNS. This suggests that CB receptors located in the CNS or periphery could mediate the cannabinoid-induced anti-inflammatory actions. The present study is the first to demonstrate that the CB_1/CB_2 receptor agonist WIN55212-2 reduced the severity of DSS- and TNBS-induced colitis. Furthermore, SAB378 had no effect on the degree of colitis induced by DSS or TNBS, suggesting that activation of peripheral cannabinoid receptors alone may not be sufficient to afford protection against either DSS- or TNBS-induced inflammation in the mouse colon. Studies investigating colitis after either chemical ablation of capsaicin-sensitive primary afferents or surgical vagotomy have revealed that these nerves play a protective role on DSS- and TNBS-induced colitis in rodents (McCafferty et al., 1997; Ghia et al., 2007). However, other groups have suggested that these neurons are essential for the induction of these types of experimental colitis in rats such that destruction of the nerves results in a less severe inflammatory outcome (Kihara et al., 2003; Fujino et al., 2004). It has been shown that cannabinoid agonists block the production of neutrophil chemoattractants and prevent the migration of neutrophils into the peritoneal cavity in a mouse model of peritonitis via central CB_1, and not CB_2, receptors (Smith et al., 2001). The present study suggests that activation of central cannabinoid receptors may be required for the anti-inflammatory actions of cannabinoid agonists in both DSS and TNBS colitis. Whether the activation of only central receptors could protect against colitis or whether dual activation of receptors in both the CNS and periphery is required remains to be determined. Our findings demonstrate that the higher dose of SAB378 (1.0 mg/kg) showed a nonsignificant trend to improve colitis, which may suggest that peripheral CB receptors could play a role in the mediation of anti-inflammatory actions of cannabinoids. However, investigating this would prove problematic because of the inhibitory actions that higher doses of SAB378 would exert on GI transit. In the present study, daily administration of SAB378 (0.1 mg/kg) further reduced body weight in mice treated with DSS, while having no effect on the degree of inflammation in this model of colitis. Likewise, subchronic daily treatment of the cannabinoid agonist HU210 [(6a*R*,

10a*R*)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol] has been shown to reduce body weight, at doses that do not affect food intake, in rats (Giuliani et al., 2000). The present study may have revealed a biphasic action of daily SAB378 administration on body weight in mice that is not related to the inflammatory state of the GI tract, because the effect was not observed at a higher dose (1.0 mg/kg) and when administered acutely during the TNBS colitis study.

In conclusion, using SAB378, a cannabinoid receptor agonist whose action is restricted to the periphery, we show that although the actions of cannabinoid receptor activation to slow GI motility may be peripherally mediated, and thus may be of therapeutic value, the anti-inflammatory effects of cannabinoid agonists on experimental colitis may require central cannabinoid receptor activation.

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