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ARTICLE

Heterogeneity of cannabinoid ligand-induced modulations in intracellular Ca^{2+} signals of mouse pancreatic acinar cells in vitro

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We recently reported that a CB_2R agonist, GW405833 (GW), reduced both the ACh-induced Ca^{2+} oscillations and the L-arginine-induced Ca^{2+} signal enhancement in mouse pancreatic acinar cells, suggesting that GW-induced inhibition may prevent the pathogenesis of acute pancreatitis. In this study, we aim to evaluate the effects of other cannabinoid ligands on Ca^{2+} signaling in acinar cells. Patch-clamp whole-cell recordings were applied to measure ACh-induced intracellular Ca^{2+} oscillations in pancreatic acinar cells acutely dissociated from wild-type (WT), CB_1R knockout (KO), and CB_2R KO mice, and the pharmacological effects of various cannabinoid ligands on the Ca^{2+} oscillations were examined. We found that all the 8 CB_2R agonists tested inhibited ACh-induced Ca^{2+} oscillations. Among them, GW, JWH133, and GP1a caused potent inhibition with IC_{50} values of 5.0, 6.7, and 1.2 μmol/L, respectively. In CB_2R KO mice or in the presence of a CB_2R antagonist (AM630), the inhibitory effects of these 3 CB_2R agonists were abolished, suggesting that they acted through the CB_2Rs. The CB_1R agonist ACEA also induced inhibition of Ca^{2+} oscillations that existed in CB_1R KO mice and in the presence of a CB_1R antagonist (AM251), suggesting a non-CB_1R effect. In WT, CB_1R KO, and CB_2R KO mice, a nonselective CBR agonist, WIN55,212-2, inhibited Ca^{2+} oscillations, which was not mediated by CB_1Rs or CB_2Rs. The endogenous cannabinoid substance, 2-arachidonoylglycerol (2-AG), did not show an inhibitory effect on Ca^{2+} oscillations. In conclusion, CB_2R agonists play critical roles in modulating Ca^{2+} signals in mouse pancreatic acinar cells, while other cannabinoid ligands modulate Ca^{2+} oscillations in a heterogeneous manner through a CB receptor or non-CB-receptor mechanism.

Keywords: cannabinoid; CB_2 receptor; CB_1 receptor; endocannabinoid; Ca^{2+} oscillations; pancreatic acinar cells; patch-clamp

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INTRODUCTION

Cannabis is a common cash crop that can be used for textiles, raw materials, and food. It is also a herbal medicine whose extract can be used as an analgesic. It is considered a drug and is regulated in many countries. At least 4% of adults in the world use cannabis each year, making it one of the most commonly used illicit drugs in the world.

Cannabinoid receptors are divided into two categories, named cannabinoid type 1 receptor (CB_1R) and cannabinoid type 2 receptor (CB_2R), which are both G protein-coupled receptors [1]. The traditional view holds that CB_1R is mainly expressed in central neurons and in small amounts in peripheral tissues and cells, while CB_2R is mainly expressed in peripheral immune cells and the hematopoietic system.

In pancreatic tissues, both CB_1R and CB_2R messenger RNAs (mRNA) are expressed, suggesting that cannabinoid receptors are involved in the regulation of pancreatic function [2, 3]. Additionally, a recent study showed that in animal models of pancreatitis, CB_2R mRNA and protein levels are changed in pancreatic acinar cells and CB_2R agonists can reduce the pathological changes in acinar cells [2, 3]. In acutely dissociated mouse pancreatic acinar cells, we reported that the selective CB_2R agonist GW405833 (GW) inhibited both the acetylcholine (ACh)-induced Ca^{2+} oscillations and the *L*-arginine-induced elevation of intracellular Ca^{2+} oscillations, indicating that CB_2R plays important roles in regulating the physiology and pathophysiology of pancreatic acinar cells [4].

Many synthetic and endocannabinoid substances show a variety of pharmacological effects. Accumulating lines of evidence suggest that the cannabinoid ligands modulate CB signaling in a complex way, including through diverse targets, signaling pathways, and heterogeneous effects, and through CB receptor (CB_1R and CB_2R) and non-CB receptor mechanisms. However, the pharmacological effects of various cannabinoid ligands on intracellular Ca^{2+} signaling in pancreatic acinar cells have not been well studied. The aim of this study was to detect the pharmacological effects of various cannabinoid ligands, including eight commercially available CB_2R agonists, one CB_1R agonist (arachidonyl-2'-chloroethylamide (ACEA)), a nonspecific CB receptor agonist (WIN55,212-2), and an endocannabinoid substance (2-arachidonoylglycerol (2-AG)), on the ACh-induced Ca^{2+} oscillations in acutely isolated pancreatic acinar cells from wild-type

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(WT), CB_1R knockout (KO), and CB_2R KO mice. The Ca^{2+} oscillations were determined by measuring the Ca^{2+}-activated Cl^- currents using patch-clamp whole-cell recordings.

MATERIALS AND METHODS

All experimental protocols were approved by and performed in accordance with the guidelines set by the animal care and use and ethical committees at the Barrow Neurological Institute and the First Affiliated Hospital of Zhengzhou University (Zhengzhou, He-nan, China).

Mouse pancreatic acinar cell preparation

The mice used for this study were adult (4–6 months old) male CD_1 mice (Charles River Laboratories International, Inc., Wilmington, MA, USA). Additionally, C57BL/6J WT, CB_1R KO [5], and CB_2R KO mice [6] of similar ages and gender on a C57BL/6J genetic background were used to determine the targets of cannabinoid ligands. Pancreatic cells were acutely isolated as previously described [7–9]. In brief, pancreatic glands were taken from isoflurane-anesthetized mice, and fragments of the pancreatic tissue were minced and digested using collagenase (200 U/mL, 25–30 min, 37 °C; Wako Pure Chemicals, Osaka, Japan) in the presence of 1 mM Ca^{2+}. After collagenase digestion, the cell suspension was gently pipetted to obtain further separation of the cells and then washed with physiological saline. A 100 μL volume of cell suspension was then poured into extracellular solution in a 2 mL experimental bath solution. The isolated cells usually adhered to the bottom within 15–20 min and were used for recording within 3 h after preparation. All experiments were performed at room temperature (22 ± 1 °C).

Conventional patch-clamp whole-cell recordings

Conventional patch-clamp whole-cell recording was used to record the Ca^{2+}-activated Cl^- currents for monitoring intracellular Ca^{2+} oscillations, as reported previously [8, 9]. Recording pipettes were made from borosilicate glass capillaries. They had a resistance of 3–5 MΩ when filled with pipette solution. After a GΩ seal was established between the cell membrane and the pipette, a whole-cell configuration was achieved by brief negative suction. Transmembrane currents were recorded with a patch-clamp amplifier (Axopatch 200B; Molecular Devices, Sunnyvale, CA, USA) at a holding potential (V_H) of −30 mV. In this study, the series resistance was not compensated.

Solution and chemicals

The standard extracellular solution contained the following (in mM): 140 NaCl, 1.0 $CaCl_2$, 4.7 KCl, 1.13 $MgCl_2$, 10 glucose, and 10 HEPES, adjusted to pH 7.2 with NaOH. The pipette solution contained the following (in mM): 140 KCl, 1.13 $MgCl_2$, 5 Na_2ATP, 0.24 EGTA, 10 glucose, and 10 HEPES, pH 7.2. The drugs used in this study were GW, GP1a, JWH133, SER601, CB65, JWH015, Hu308, L759656 ACEA, WIN55,212-2, and 2-AG (Fig. 1), which were purchased from Tocris Bioscience (Minneapolis, MN, USA). ACh was purchased from Sigma-Aldrich (St Louis, MO, USA). A stream of the standard extracellular solution was continuously perfused over the cell during recording. A computer-controlled U-tube system was used for drug application [10]. For intracellular drug application, the drug was added to the pipette solution, and the establishment of a whole-cell configuration allowed the drug to diffuse into the recorded cell.

Statistical analysis

For patch-clamp experiments, the Ca^{2+}-activated Cl^- current responses in dissociated mouse pancreatic acinar cells (Fig. 2) were presented as the current charge (current area· Cm^{-1} min^{-1}), and then the drug-induced changes were compared to the baseline level of charge (induced by ACh). All data are presented as the mean ± SEM. When data were obtained from the same recorded cell and the changes in ACh response were compared before and after drug exposure, paired Student's *t*-test was used. To analyze the effects of CB ligands on different groups of cells (eg, WT, CB_1R, and CB_2R KO mice), one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used.

RESULTS

Effects of CB_2R agonists GW, JWH133, GP1a on ACh-induced Ca^{2+} oscillations

To determine the effects of CB_2R agonists on intracellular Ca^{2+} signals, we examined the effect of three CB_2R agonists with similar

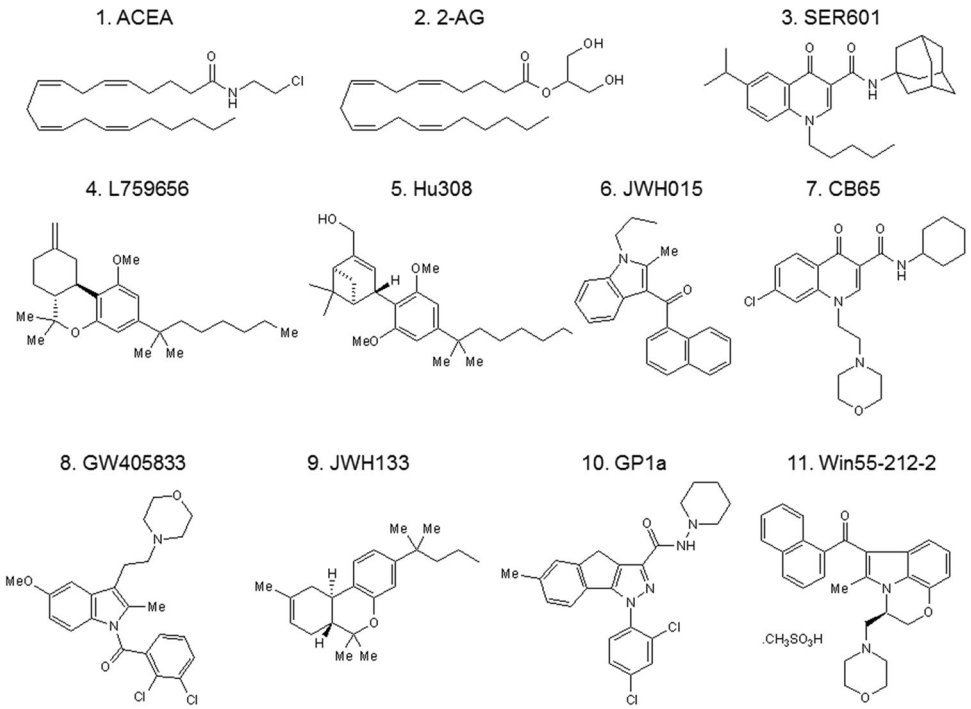

Fig. 1 Chemical structures of 11 tested cannabinoid ligands

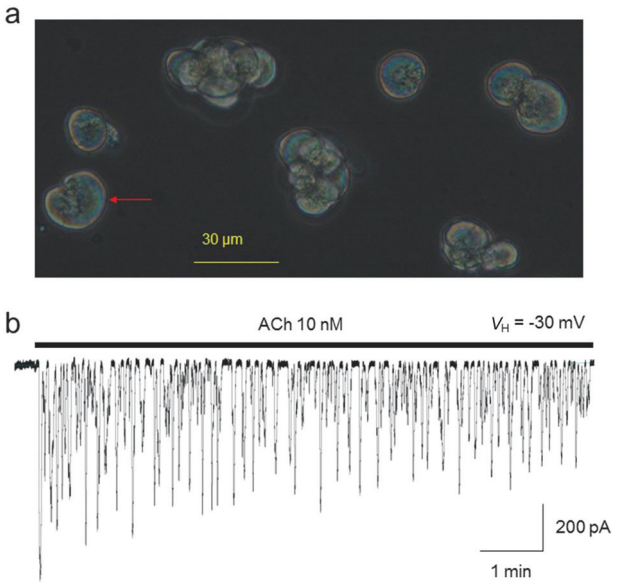

Fig. 2 **a** Photograph of freshly dissociated mouse pancreatic acinar cells. Typical acinar cells have a kidney shape (indicated by red arrow). **b** Representative trace of ACh-induced Ca^{2+} oscillations by measuring Ca^{2+}-dependent Cl^- current oscillations in patch-clamp whole-cell recording

chemical structures (Fig. 1) on ACh-induced Ca^{2+} oscillations. The results showed that the three tested CB_2R agonists (at 10 μM concentration), GW, JWH133, and GP1a, showed marked inhibition. As shown in Fig. 3a–c, 10 μM GW, JWH133, or GP1a significantly inhibited the 10 nM ACh-induced Ca^{2+} oscillations. Statistical analysis showed that after exposure of WT mice to GW, JWH133, and GP1a, the normalized net charges of 10 nM ACh-induced Ca^{2+} oscillations were reduced to 23% ± 4.4% ($P < 0.001$, $n = 7$), 26% ± 4.2% ($P < 0.001$, $n = 9$), and 4.6% ± 4.2% ($P < 0.001$, $n = 6$), respectively (Fig. 3d). In CB_2R KO mice (Fig. 3e) or in the presence of the CB_2R antagonist AM630 (Fig. 3g), the 10 μM GW-, JWH133-, and GP1a-induced inhibition of 30 nM ACh-induced Ca^{2+} oscillations was abolished. The concentration–inhibition relationship curves for these three agonists were measured, showing half-maximal inhibitory concentration (IC_{50}) values of 5.0, 6.7, and 1.2 μM for GW-, JWH133-, and GP1a-induced inhibition, respectively (Fig. 3f). These results indicate that the CB_2R agonists GW, GP1a, and JWH133 potently inhibit the intracellular Ca^{2+} signal through CB_2Rs of mouse pancreatic acinar cells.

Effects of CB_2R agonists SER601, CB65, Hu308, L759656, and JWH015 on ACh-induced Ca^{2+} oscillations

We tested five other CB_2R agonists, SER601, CB65, Hu308, L759656, and JWH015, that possess different chemical structures (Fig. 1). The results showed a mild reduction in Ca^{2+} oscillations for all tested agonists (Fig. 4a–e). Statistical analysis (Fig. 4f) showed that after exposure to 10 μM SER601, CB65, Hu308, L759656, and JWH015, the normalized net charges of 10 nM ACh-induced Ca^{2+} oscillations were reduced ($F_{30,6} = 9.59$, $P < 0.0001$). Tukey's *post hoc* comparison between baseline and each CB_2R agonist tested showed the effects of SER601 (88.1% ± 5.1%, $P < 0.001$), CB65 (85.9% ± 5.5%, $P < 0.01$), Hu308 (88.0% ± 4.0%, $P < 0.001$), L759656 (96.4% ± 2.09%, $P > 0.05$), and 89.0% ± 6.4%, $P < 0.01$). These results demonstrate that four of the five tested CB_2R agonists showed lower affinity/potency of inhibition of intracellular Ca^{2+} signaling in pancreatic acinar cells, while L759656 did not show a significant effect.

Effect of the CB_1R agonist on ACh-induced Ca^{2+} oscillations

It has been reported that both CB_1R and CB_2R are expressed in mouse pancreatic tissue [3], but in our acute dissociated mouse pancreatic acinar cells, the CB_1R mRNA was not detected [4]. Here, we tested the effect of CB_1R agonists on ACh-induced Ca^{2+} oscillations. As shown in Fig. 5a, the CB_1R agonist ACEA (10 μM) reversibly reduced 30 nM ACh-induced Ca^{2+} oscillations. Considering that ACEA was solubilized with ethanol (10 μM ACEA = 7.3 mM ethanol), we examined the effect of 7.3 mM ethanol on ACh-induced Ca^{2+} oscillations and found a similar inhibitory effect on 30 nM ACh-induced Ca^{2+} oscillations (Fig. 5b). Furthermore, we examined the inhibitory effect of ACEA on the ACh-induced Ca^{2+} oscillations in the presence of the CB_1R antagonist AM251 (Fig. 5c) or in the pancreatic acinar cells isolated from either CB_1R KO (Fig. 5d) or CB_2R KO (Fig. 5e) mice and found a similar inhibition. One-way ANOVA showed that there was no significant difference in ACEA-induced inhibition among the five tested groups ($P > 0.05$, Fig. 5f). These results suggest that the inhibitory effect of ACEA on the ACh-induced Ca^{2+} oscillations is not mediated through either CB_1R or CB_2R.

Effect of the nonselective cannabinoid receptor agonist WIN55,212-2 on the ACh-induced Ca^{2+} oscillations

To further clarify the regulatory role of cannabinoid CB_1 and CB_2 receptors, we tested the effect of a classic synthetic nonselective CBR agonist, WIN55,212-2, since this compound has been widely used to test the effects of the cannabinoid. The results showed that WIN55,212-2 inhibited the ACh-induced Ca^{2+} oscillations in a concentration-dependent manner, with an IC_{50} value of 1.1 μM (Fig. 6a–d). Statistical analysis (Fig. 6d) showed that the normalized net charge was reduced to 7% ± 3.5% ($P < 0.001$, $n = 5$) on 30 nM ACh-induced Ca^{2+} oscillations after the addition of 10 μM WIN55,212-2. Then, we compared the effects of WIN55,212-2 (10 μM) on 30 nM ACh-induced Ca^{2+} oscillations in WT, CB_1R, and CB_2R KO mice. The results showed that WIN55,212-2 (10 μM) inhibited the 30 nM ACh-induced Ca^{2+} oscillations in all WT (Fig. 6e), CB_1R KO (Fig. 6f), and CB_2R KO mice (Fig. 6g). Statistical analysis showed that the rates of inhibition (the currents of 1-drug/baseline) by WIN55,212-2 (10 μM) of the normalized net charge of the ACh-induced Ca^{2+} oscillations were 92.5% ± 3.5% ($P < 0.001$, $n = 6$), 92.5% ± 3.1% ($P < 0.001$, $n = 6$), and 90.7% ± 4.6% ($P < 0.001$, $n = 8$), respectively, after addition of 10 μM WIN to pancreatic acinar cells isolated from WT, CB_1R KO and CB_2R KO mice (Fig. 6h). These results suggest that the inhibition of Ca^{2+} oscillations by WIN55,212-2 is not mediated by CB_1R or CB_2R.

Effect of the endocannabinoid substance 2-AG on ACh-induced Ca^{2+} oscillations

Lastly, we examined the effects of an endogenous cannabis-like substance, 2-AG, on 30 nM ACh-induced Ca^{2+} oscillations in pancreatic acinar cells dissociated from WT mice. As shown in Fig. 7, 2-AG at either 10 μM (Fig. 7a) or 100 μM (Fig. 7b) failed to affect the ACh-induced Ca^{2+} oscillations (Fig. 7c).

DISCUSSION

In this study, we have systematically examined the pharmacological effects of cannabinoid ligands on ACh-induced Ca^{2+} oscillations in single pancreatic acinar cells acutely dissociated from WT, CB_1R, and CB_2R KO mice. We found heterogeneous effects of different cannabinoid ligands on Ca^{2+} oscillations through the different targets. First, all tested CB_2R ligands showed inhibitory effects with different efficiencies. Three higher-efficiency agonists, GW, JWH133, and GP1a, showed remarkable inhibition of the ACh-induced Ca^{2+} oscillations. Based on the IC_{50} values of the concentration–inhibition curves of these three agonists, the order of affinity was GP1a > GW > JWH133 for

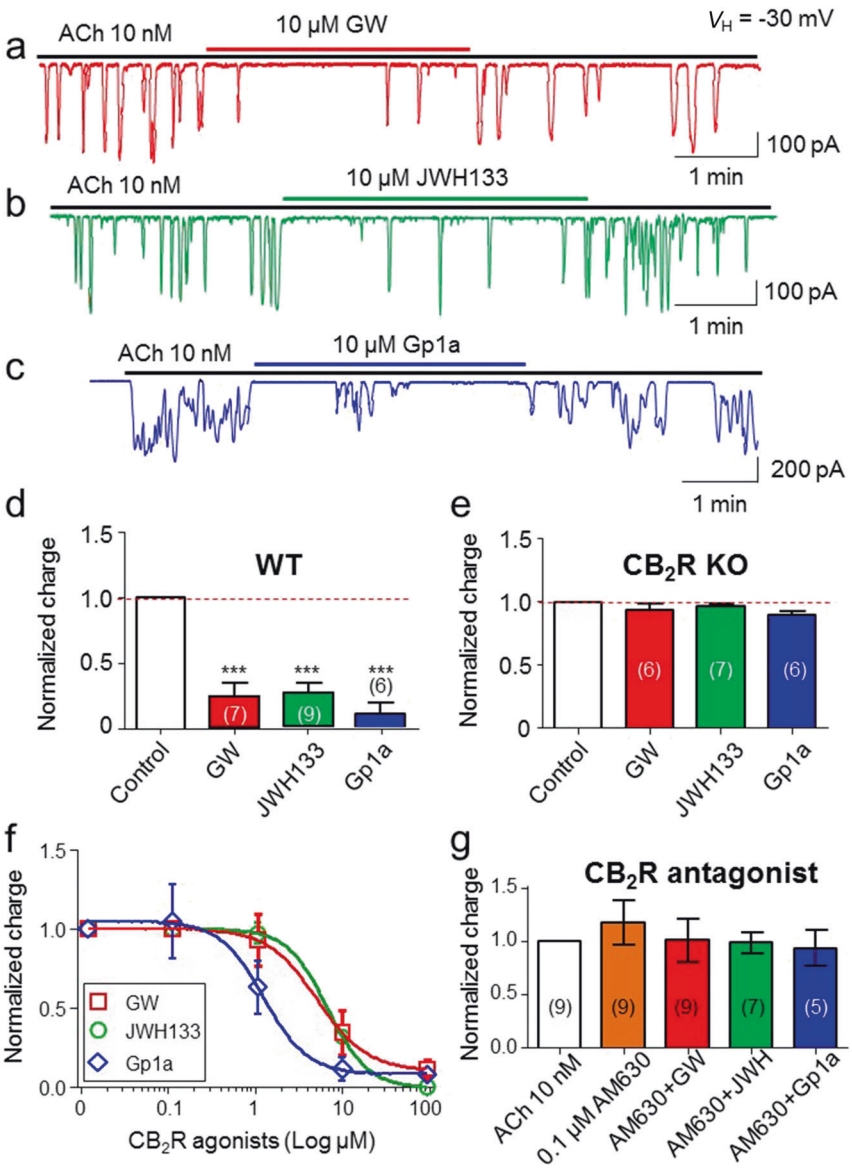

Fig. 3 Effects of the CB_2R agonists GW, JWH133, and GP1a on the ACh-induced Ca^{2+} oscillations. **a**–**c** Representative traces of the effects of 10 μM GW (**a**), JWH133 (**b**) and GP1a (**c**) on 10 nM ACh-induced Ca^{2+} oscillations in pancreatic acinar cells. **d** The bar graph summarizes the effects of three CB_2R agonists on the ACh-induced Ca^{2+} oscillations. ***$P < 0.001$. **e** Similar experiments were done as in (**d**) but in CB_2R-KO mice and showed that the above three CB_2R agonists did not affect the ACh-induced Ca^{2+} oscillations in CB_2R-KO mice. **f** Concentration–inhibition relationship curves of GW, JWH133, and GP1a. **g** The effects of a CB_2R antagonist (0.1 μM AM630) on the GW-, JWH133-, and GP1a-induced inhibition of the ACh-induced Ca^{2+} oscillations. In this and all following figures, the vertical column represents an averaged mean of total charge (current area/Cm per 2 min), and the vertical bars indicate the standard error

inhibiting the ACh-induced Ca^{2+} oscillations. The inhibitory effects of these CB_2R ligands were eliminated in the acinar cells dissociated from CB_2R KO mice or in the presence of a CB_2R antagonist (AM630), which suggests that the inhibition is mediated through the CB_2Rs. Then, a selective CB_1R agonist, ACEA, also showed inhibition of the ACh-induced Ca^{2+} oscillations. However, this inhibition was still maintained in the presence of CB_1R antagonists (AM251) or in the acinar cells dissociated from either CB_1R KO or CB_2R KO mice. This suggests that ACEA inhibits ACh-induced Ca^{2+} oscillations through a non-CBR mechanism. Since ACEA was dissolved in ethanol, we tested the effects of 7.3 mM (equal to 10 μM ACEA) ethanol and found a similar inhibition. Furthermore, a commonly used, nonselective CBR ligand, WIN55,212-2, showed significant inhibition of the ACh-induced Ca^{2+} oscillations, with an IC_{50} value of 1.1 μM. Unexpectedly, the WIN55,212-2-induced inhibition still occurred in the acinar cells dissociated from either CB_1R KO or CB_2R KO mice, suggesting that WIN55,212-2-induced inhibition of ACh-induced Ca^{2+} oscillations is not mediated by CB_1R or CB_2R. Finally, an endogenous cannabis-like substance, 2-AG, did not alter the ACh-induced Ca^{2+} oscillations, even at a concentration of 100 μM. Collectively, we have profiled the heterogeneous nature of the pharmacological properties of different CBR ligands in the intracellular Ca^{2+} signals of mouse pancreatic acinar cells. Our results show that cannabinoid ligands modulate cellular Ca^{2+} signals through heterogeneous pathways, in which CB_2R ligands inhibit the ACh-induced Ca^{2+} oscillations by targeting CB_2Rs, while other ligands, such as ACEA and WIN55,212-2, modulate Ca^{2+} oscillations that are not mediated through the CBRs. This study provides new insights into cannabinoid modulations of the

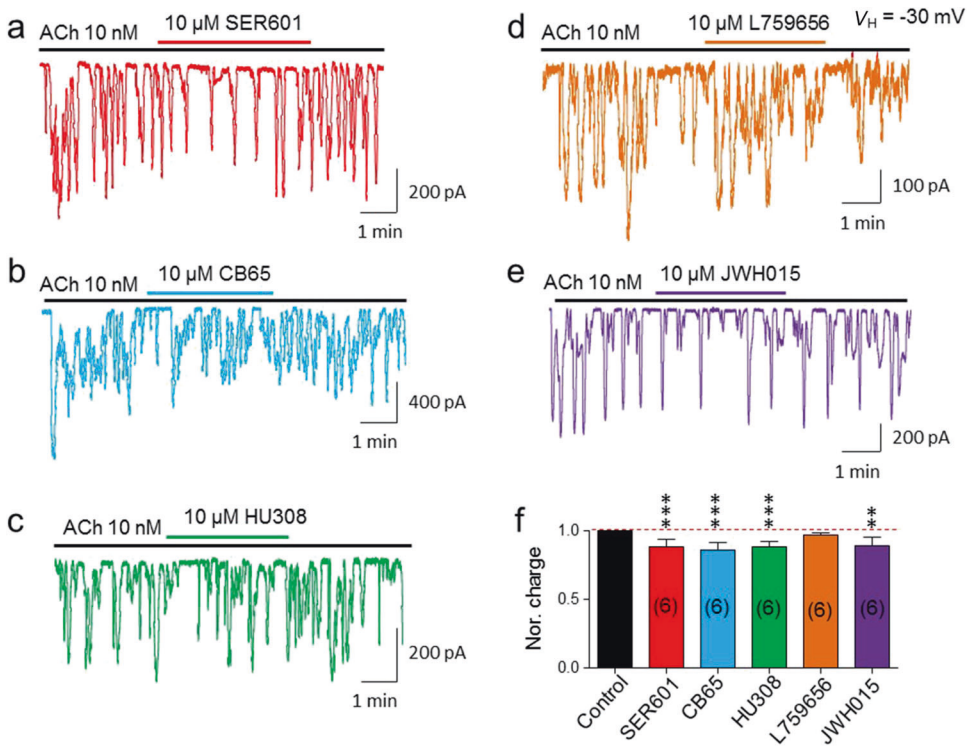

Fig. 4 CB_2R agonists SER601, CB65, Hu308, L759656, and JWH015 show weak inhibition of ACh-induced Ca^{2+} oscillations. **a**–**e** Representative traces of the effects of 10 μM SER601 (**a**), CB65 (**b**), Hu308 (**c**), L759656 (**d**), and JWH015 (**e**) on 10 nM ACh-induced Ca^{2+} oscillations in pancreatic acinar cells of WT mice. **f** Statistical analysis shows that these five CB_2R agonists with different chemical structures mildly inhibit the ACh-induced Ca^{2+} oscillations. $^{**}P < 0.01$, $^{***}P < 0.001$

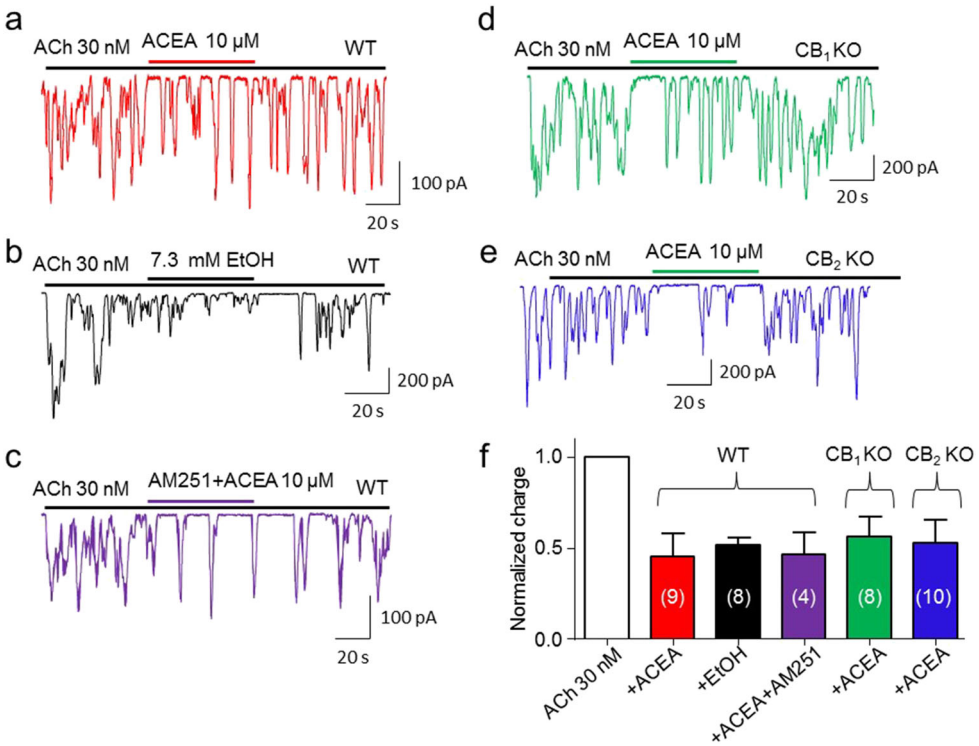

Fig. 5 Effects of CB_1R agonist (ACEA, 10 μM) on ACh-induced Ca^{2+} oscillations. **a** A typical case of 10 μM ACEA (dissolved by 7.3 mM ethanol)-induced reduction in ACh-induced Ca^{2+} oscillations in WT mice. **b** A typical trace of the effects of 7.3 mM ethanol (as a control) on the ACh-induced Ca^{2+} oscillations. **c** A typical trace of the effects of the CB_1R antagonist AM251 (1 μM) on 10 μM ACEA-induced inhibition of the ACh-induced Ca^{2+} oscillations. **d** A typical trace of the effects of ACEA on the ACh-induced Ca^{2+} oscillations in CB_1R KO mice. **e** A typical trace of the effects of ACEA on the ACh-induced Ca^{2+} oscillations in CB_2R KO mice. **f** Statistical analysis shows similar levels of inhibition of the ACh-induced Ca^{2+} oscillations in all tested groups. One-way ANOVA shows no significant difference among the effects of these CB_2R agonists

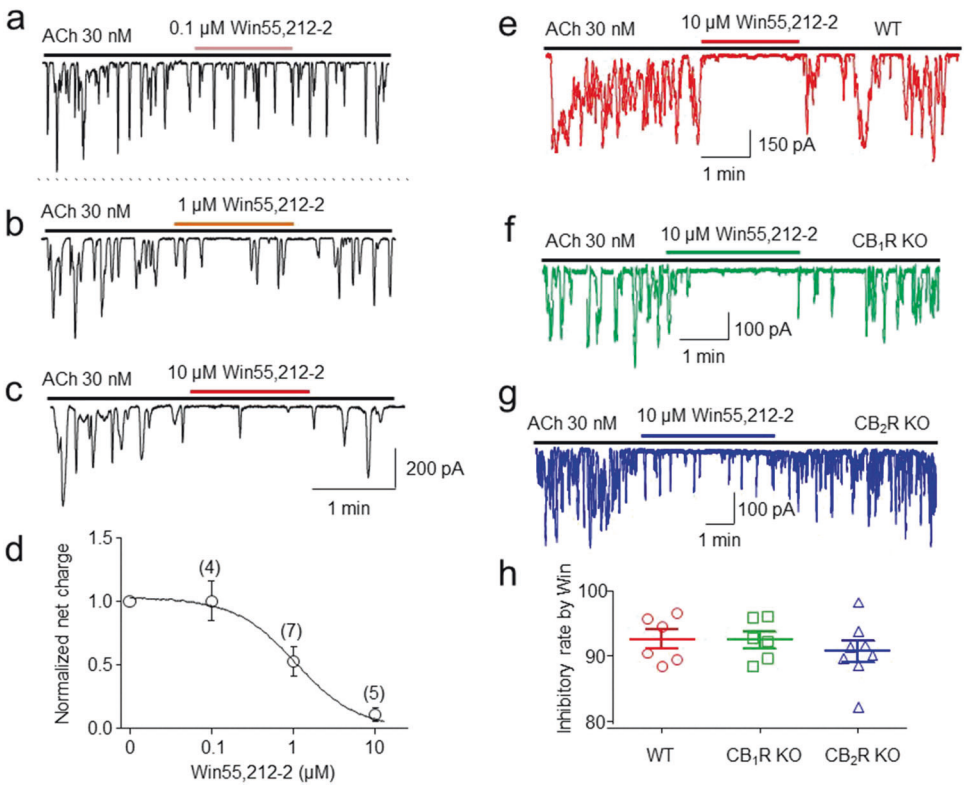

Fig. 6 Effect of a nonselective CBR agonist WIN55,212-2 (10 μM) on 30 nM ACh-induced Ca^{2+} oscillations. **a**–**c** Typical traces of the WIN55,212-2-induced inhibition of the ACh-induced Ca^{2+} oscillations in a concentration-dependent manner in WT mice, which formed a sigmoidal concentration–inhibition relationship curve (**d**). In the acinar cells prepared from WT (**e**), CB_1R KO (**f**), or CB_2R KO (**g**) mice, WIN55,212-2 reduced the ACh-induced Ca^{2+} oscillations. **h** Statistical analysis (one-way ANOVA) indicates that there are no significant differences among the inhibitory effects of WIN55,212-2 on the Ca^{2+} oscillations of the acinar cells prepared from WT, CB_1R KO, and CB_2R KO mice

Ca^{2+} signal in mouse pancreatic acinar cells and suggests that carefully selected CB_2R agonists should be considered as novel therapeutic compounds for treating acute pancreatitis.

CB_2R is the major target to mediate the cannabinoid modulations in the ACh-induced Ca^{2+} oscillations in mouse pancreatic acinar cells

Cannabinoid receptors are divided into two subtypes, CB_1R and CB_2R. CB_2R has been called the "peripheral" cannabinoid receptor because it is mainly expressed in peripheral tissues, including immune cells, cardiomyocytes, gastrointestinal cells, and hepatocytes [11–17]. In pancreatic acinar cells, CB_2R protein expression has been found in rodents using immunohistochemical staining and Western blotting [3, 18]. In mouse pancreatic tissues, CB_1R and CB_2R mRNAs have been identified using reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical staining [3]. Functionally, we examined the roles of CB_2Rs in the modulation of the ACh-induced Ca^{2+} oscillation in mouse pancreatic acinar cells, a well-established cell model for the study of intracellular Ca^{2+} signals [19]. Using patch-clamp whole-cell recording to measure Ca^{2+}-activated Cl^-currents, we found that all eight commercially available CB_2R agonists showed significant inhibition of Ca^{2+} oscillations. Three of them, GW, JWH133, and GP1a, showed dramatic inhibition, and this inhibition was mediated through CB_2R. These results support previous reports that CB_2R agonists play critical roles in the prevention of acute pancreatitis pathology [3] and the reduction of intracellular Ca^{2+} signals [18].

Effects of CB_1R agonists on the ACh-induced Ca^{2+} oscillations in mouse pancreatic acinar cells

Whether mouse pancreatic acinar cells express CB_1Rs is unknown. Both CB_1R and CB_2R mRNA and proteins have been detected in pancreatic tissue [3, 18]. Using real-time RT-PCR, our recent work detected CB_2R but not CB_1R mRNA in freshly isolated mouse pancreatic acinar cells [4]. Therefore, in this study, we examined the effects of a CB_1R agonist on the ACh-induced Ca^{2+} oscillations in isolated single mouse pancreatic acinar cells. Interestingly, we found that a CB_1R selective agonist, ACEA, also inhibited the ACh-induced Ca^{2+} oscillations. To determine whether the effect of ACEA was mediated by CB_1Rs, we tested the effect of a CB_1R antagonist (AM251, 10 μM) on the ACEA-induced inhibition and found that AM251 failed to prevent ACEA's effect. Moreover, we tested the effects of ACEA on the Ca^{2+} oscillations in the acinar cells dissociated from CB_1R KO and WT mice and found similar inhibition by ACEA in these two groups. These results suggest that the ACEA-induced inhibition of ACh-induced Ca^{2+} oscillations is not mediated by CB_1R. ACEA also inhibited Ca^{2+} oscillations in CB_2R KO mice, suggesting that the CB_2R is also not the target for the ACEA. Considering that ACEA was dissolved in ethanol (10 μM ACEA contained 7.3 mM ethanol), we further examined the effects of 7.3 mM ethanol and found a similar inhibition on the ACh-induced Ca^{2+} oscillations, suggesting that ACEA-induced inhibition was likely a solvent effect. Taken together, our data demonstrate that the ACEA-induced inhibition of cellular Ca^{2+} oscillations is achieved through a non-CBR mechanism. This functional study supports our previous work, in which we failed to detect CB_1R mRNA expression in mouse pancreatic acinar cells [4]. The reason for the differences between previous reports and ours may be the use of different preparations. Previous reports that tested CB_1R and CB_2R mRNA expression used pancreatic tissue [3], while we used dissociated pancreatic acinar cells [4]. In pancreatic tissue, there are other types of cells, such as beta-cells, which have been reported to express both CB_1R and CB_2R [3, 18].

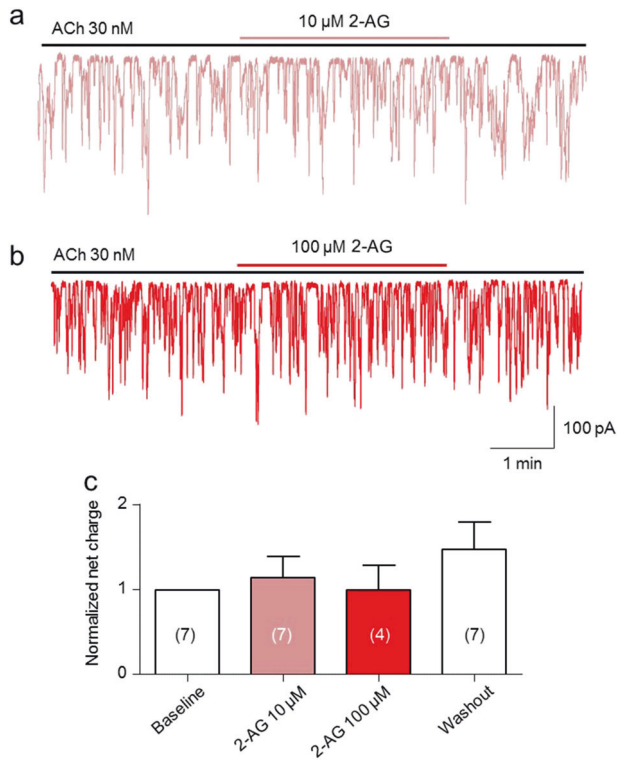

Fig. 7 Effect of the endocannabinoid substance 2-AG on 30 nM ACh-induced Ca^{2+} oscillations. **a** A typical trace of the effects of 2-AG (10 μM) on 30 nM ACh-induced Ca^{2+} oscillations. **b** A typical trace of the effects of 2-AG (100 μM) on 30 nM ACh-induced Ca^{2+} oscillations. **c** The bar graph summarizes the effects of 2-AG on Ca^{2+} oscillations

Effects of nonspecific CBR ligands on the ACh-induced Ca^{2+} oscillations

In the cannabinoid signaling field, many studies have used nonspecific CBR ligands such as WIN55,212-2 and endocannabinoid substances such as 2-AG. These ligands nonselectively activate both CB_1Rs and CB_2Rs. Thus, we tested the effects of WIN55,212-2 and 2-AG on the ACh-induced Ca^{2+} oscillations. Interestingly, we found that WIN55,212-2 inhibited Ca^{2+} oscillations in a non-CBR way (Fig. 6), while 2-AG did not show a significant effect on the Ca^{2+} oscillations, even at a high concentration (Fig. 7b, c). Although the precise mechanisms of these diverse effects by cannabinoid ligands on the Ca^{2+} oscillations in mouse pancreatic acinar cells are still unclear, our findings suggest the heterogeneous and complex ways that cannabinoid ligands modulate intracellular Ca^{2+} signals. Emerging evidence shows that there are complex relationships between cannabinoid receptors and intracellular Ca^{2+} signals in different types of cells. For example, the activation of CB_1R or CB_2R increases intracellular Ca^{2+} levels in endothelial cells, submandibular acinar cells, canine kidney cells, and bladder cancer cells [20–23], while the activation of CB_1R or CB_2R reduces glucose-induced intracellular Ca^{2+} oscillations and insulin release in pancreatic beta-cells [24, 25].

Clinical significance of CB_2R agonists in the modulation of Ca^{2+} signals in pancreatic acinar cells

Acute pancreatitis is an inflammatory disease with complex pathogenesis requiring immediate medical treatment [26, 27]. In clinical practice, there are still no effective drugs for the specific treatment of acute pancreatitis [26]. Pancreatic acinar cells are exocrine pancreatic units that synthesize, store, and secrete inactive digestive enzymes into the lumen of acinar cells. The activity of pancreatic acinar cells is strictly regulated by the secretagogues ACh and cholecystokinin (CCK), both of which act on specific membrane receptors (muscarinic and CCK receptors, respectively) and then increase the Ca^{2+} levels in the cytoplasm. If the intracellular Ca^{2+} has been maintained at a high concentration, the intracellular signal transduction is disrupted, harming the cells and resulting in acute pancreatitis. Recent studies have shown that the early pathological changes in acute pancreatitis are caused by an abnormal increase in Ca^{2+} levels in acinar cells. The sustained increase in intracellular Ca^{2+} levels can trigger digestive enzymes, resulting in inflammation and necrosis. Drugs that block the accumulation of calcium store-operated Ca^{2+} channels will prevent the sustained increase in intracellular Ca^{2+} levels and protease activation [28]. In an animal model of acute pancreatitis, activation of CB_2Rs (but not CB_1Rs) in pancreatic acinar cells can prevent pathological changes caused by cerulein or *L*-arginine [3]. In our previous study, we reported that the CB_2R agonist GW reduced ACh-induced Ca^{2+} oscillations, eliminated *L*-arginine-induced enhancement of Ca^{2+} oscillations, and prevented the *L*-arginine-induced increases in serum amylase and lung myeloperoxidase levels in acute pancreatitis [4]. These results suggest that CB_2R agonists rather than CB_1R agonists may be potential novel therapeutic agents for the prevention and treatment of acute pancreatitis.

In conclusion, our study profiles the pharmacological effects of cannabinoid ligands on the intracellular Ca^{2+} signals in mouse pancreatic acinar cells. It confirms that modulation by CBR ligands can be accomplished through CB_2R as well as through non-CBR pathways. In consideration of the heterogeneity of the regulatory process, we should be cautious when selecting CBR ligands as potential drugs for the treatment of acute pancreatitis.

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AUTHOR CONTRIBUTIONS

K.-k.X. performed patch-clamp recording, data analysis, made figures, and wrote initial manuscript; J.-x.S. performed some experiments, data analysis, and wrote part manuscript; Z.-b.H. performed some experiments and analysis data; H.-m.S. performed experiments and data analysis; M.G. performed experiments and data analysis; D.-j.C. performed data analysis; S.-j.Z. designed experiments and revised manuscript; J.W. designed experiments, data analysis, made and revised figures, and wrote and revised the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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