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WIN55212-2 Modulates Intracellular Calcium via CB_1 Receptor-Dependent and Independent Mechanisms in Neuroblastoma Cells

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Abstract: The CB_1 cannabinoid receptor (CB_1R) and extracellular calcium (eCa^{2+})-stimulated Calcium Sensing receptor (CaSR) can exert cellular signaling by modulating levels of intracellular calcium ($[Ca^{2+}]_i$). We investigated the mechanisms involved in the ($[Ca^{2+}]_i$) increase in N18TG2 neuroblastoma cells, which endogenously express both receptors. Changes in $[Ca^{2+}]_i$ were measured in cells exposed to 0.25 or 2.5 mM eCa^{2+} by a ratiometric method (Fura-2 fluorescence) and expressed as the difference between baseline and peak responses ($\Delta F_{340/380}$). The increased ($[Ca^{2+}]_i$) in cells exposed to 2.5 mM eCa^{2+} was blocked by the CaSR antagonist, NPS2143, this inhibition was abrogated upon stimulation with WIN55212-2. WIN55212-2 increased $[Ca^{2+}]_i$ at 0.25 and 2.5 mM eCa^{2+} by 700% and 350%, respectively, but this increase was not replicated by CP55940 or methyl-anandamide. The store-operated calcium entry (SOCE) blocker, MRS1845, attenuated the WIN55212-2-stimulated increase in $[Ca^{2+}]_i$ at both levels of eCa^{2+}. Simultaneous perfusion with the CB_1 antagonist, SR141716 or NPS2143 decreased the response to WIN55212-2 at 0.25 mM but not 2.5 mM eCa^{2+}. Co-perfusion with the non-CB_1/CB_2 antagonist O-1918 attenuated the WIN55212-2-stimulated $[Ca^{2+}]_i$ increase at both eCa^{2+} levels. These results are consistent with WIN55212-2-mediated intracellular Ca^{2+} mobilization from store-operated calcium channel-filled sources that could occur via either the CB_1R or an O-1918-sensitive non-CB_1R in coordination with the CaSR. Intracellular pathway crosstalk or signaling protein complexes may explain the observed effects.

Keywords: Ca^{2+} mobilization; receptor crosstalk; cannabimimetic aminoalkylindoles

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1. Introduction

Phytocannabinoids, endocannabinoids, synthetic cannabinoids, and aminoalkylindole agonists regulate neuronal activity by activating the CB_1 receptor (CB_1R) to signal via Gi/o and other G proteins, but little is known about modulating intracellular calcium concentration ($[Ca^{2+}]_i$). Although initial studies could not detect Ca^{2+} mobilization in cultured cell models, evidence indicates that the effects of cannabinoid receptors on $[Ca^{2+}]_i$ depend on the agonist and the cell type tested. In CHO cells, the synthetic cannabinoid HU210 and its non-CB_1-binding isomer HU211 (10 μM) both induced only a non-receptor-mediated increase in $[Ca^{2+}]_i$ in untransfected, CB_1R-expressing, or CB_2R-expressing cells, using a Fura-2 method that readily detected muscarinic receptor-mediated Ca^{2+} mobilization [1,2]. In the murine neuroblastoma cell line N18TG2 endogenously expressing CB_1R (but not CB_2R), treatment with the non-selective $CB_{1/2}R$ agonists 2-arachidonoylglycerol (2-AG), CP55940, or WIN55212-2 (up to 1 μM) failed to evoke Ca^{2+} mobilization using Fluo-4

fluorescence able to detect a bradykinin-mediated response [3]. The Sugiura laboratory investigated Ca^{2+}-deprived suspensions of HL60 monocytic cells endogenously expressing CB_2R or NG108-15 neuro-glioma hybrid cells endogenously expressing CB_1R. In this setting, Ca^{2+} mobilization was stimulated by 1 mM eCa^{2+} followed by 2-AG, CP55940, or WIN55212-2 (up to 10 μM) and detected with Fura-2 [4,5]. Subsequent studies showed that the aminoalkylindole WIN55212-2, but not Δ^9-tetrahydrocannabinol (Δ^9-THC), HU210, CP55940, 2-AG, or methanandamide, increased $[Ca^{2+}]_i$ in HEK293 cells exogenously expressing CB_1R [6]. The WIN55212-2-stimulated Ca^{2+} mobilization occurred in a CB_1-dependent manner requiring $G\alpha_q$ activation and release of Ca^{2+} from thapsigargin-sensitive endoplasmic reticulum (ER) stores [6]. In addition, GPR55 and GPR18 receptors have been shown to modulate $[Ca^{2+}]_i$ in neurons and other cell types in response to lipid mediators, including atypical cannabinoids [7].

Mounting evidence supports intracellular Ca^{2+} as an important second messenger for excitable and non-excitable cells, with the inositol trisphosphate (IP_3)/Calcium signaling pathway playing a vital role in linking extracellular signals to $[Ca^{2+}]_i$ [8]. Thus, one of the receptor systems involved, the G protein-coupled calcium sensing receptor (CaSR) detects extracellular Ca^{2+} (eCa^{2+}) concentration, linking it to intracellular signaling affecting cell function [9]. The CaSR can couple to more than one type of Gα subunit and influence the properties of Gβγ signaling [10]. CaSR actions have been reported to act through Gαi, $G\alpha_q$, and Gβγ, with activation of phospholipase C, production of IP_3 through $G\alpha_q$, and Ca^{2+} release from the ER, being one of the major effects of CaSR activation [11]. In N18TG2 neuronal cells, stimulation of CaSR with the positive allosteric modulator calindol increased $[Ca^{2+}]_i$ in a response dependent on $G\alpha_{i/o}$ and modulated by $G\alpha_q$ [12]. Modulating $[Ca^{2+}]_i$ also seems dependent on PKC activity and localization [13]. It appears that the CaSR intracellular pathways activated by eCa^{2+} proceed via $G\alpha_s$ and $G\alpha_q$, whereas activation by calcimimetics occurs via $G\alpha_i$ [9].

In the present study, we aimed to explore neuronal mechanisms involved in WIN55212-2-mediated Ca^{2+} mobilization as observed by Lauckner and colleagues [6], with a focus on the extracellular $[Ca^{2+}]$ influence associated with the Sugiura procedure [4,5]. We were particularly interested in the cannabinoid receptors mediating the WIN55212-2-dependent responses in $[Ca^{2+}]_i$ and the role of CaSR activation on those responses. $[Ca^{2+}]_i$ regulation has relevant physiological significance, for example, in muscle [14] and brain tissue [15], where a role for CB_1Rs has been demonstrated. Since the CaSR monitors the extracellular Ca^{2+} environment, our studies were performed in the N18TG2 neuroblastoma cell model that endogenously expresses both CB_1R and CaSR.

2. Materials and Methods

2.1. Cells

Mouse N18TG2 neuroblastoma cells were cultured as described [16], maintained in complete media containing Dulbecco's Modified Eagle's Medium (DMEM): Ham's F-12 (1:1) supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) and 10% heat-inactivated bovine serum. Cells were grown in 75-cm^2 flasks at 37 °C in a humidified atmosphere (5% CO_2), harvested at sub-confluency, and transferred to 12 mm glass coverslips (Fisher Scientific Co., Waltham, MA, USA). At 50–75% confluence, cells were loaded for 15 min with Fura-2 (5 μM) in Krebs–Henseleit Buffer (KHB) containing (in mM) NaCl 118, KCl 4.47, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2 \cdot 2H_2O$ 0.25, glucose 5.5. Cells were incubated in two different extracellular $[Ca^{2+}]$ (eCa^{2+}) and the responses in intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) were measured.

2.2. Imaging

Coverslips were transferred to an imagining chamber on an inverted Olympus BBX51WI microscope equipped with a 40× objective, a xenon arc lamp (Sutter Instruments, Novato, CA, USA), and a manual stage, and a cooled charge-couple device (CCD) camera (Hamamatsu Orka II). For ratiometric imaging, the microscope was computer-controlled

by HCImage software (Hamamatsu Corporation, Middlesex, NJ, USA). Cells on the field were manually marked for analysis, and F_{340} and F_{380} were measured for one min. KHB containing 0.25 mM Ca^{2+} (Low eCa^{2+}) was passed through the imaging chamber for 5 min after which eCa^{2+} was changed to 2.5 mM (High eCa^{2+}) using a perfusion valve control system (VC-6, Six Channel Perfusion Valve Control Systems, Warner Instruments, Holliston, MA, USA) and cells perfused for additional 10 min. This procedure was repeated in the presence of cannabinoid receptor agonists (WIN55212-2, CP55940, or methanandamide) in KHB containing low or high eCa^{2+}. Cells were pre-incubated for 15 min for the treatments with antagonists, and the corresponding antagonists were added to the low and high eCa^{2+} solutions. Only one treatment was carried out on each coverslip used.

2.3. Drugs

The ratiometric fluorescent dye Fura-2 was purchased from Molecular Probes (Eugene, OR, USA), dissolved in dimethylsulfoxide at 1 mM, and further diluted in KHB containing 0.25 mM Ca^{2+} to a working concentration of 5 μM as described [17]. The aminoalkylindole agonist of $CB_{1/2}$ cannabinoid receptors WIN55212-2 (5 μM) [6], the prototype bicyclic non-selective $CB_{1/2}R$ agonist CP55940 (5 μM) [6], and the stable chiral analog of anandamide, a CB_1R partial agonist, methanandamide (5 μM) [6], were from Cayman Chemical Co, (Ann Arbor, MI, USA). Receptor antagonists used include the blocker of CaSR, NPS2314 (3 μM) [18], the selective store-operated calcium (SOC) channel inhibitor N-propargyl-nitrendipine (MRS1845, 10 μM) [19], the CB_1R antagonist SR141716 (1 μM) [6], and the non CB_1/CB_2 receptor blocker O-1918 (10 μM) [20], all from Cayman Chemical Co, (Ann Arbor, MI, USA). The cannabinoid compounds were stored at −20 °C as 10 mM stock solutions in ethanol. Immediately before use, an aliquot of drug stocks was air-dried and re-suspended in 0.25 mM Ca^{2+} KHB. All other chemical reagents were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.4. Data Analysis

Average changes in F_{340} and F_{380} were recorded continuously, and $[Ca^{2+}]_i$ responses were determined using the ratiometric method (ratio between F_{340} and F_{380}) [17]. Imaging measurements were repeated 5 to 7 times with a total of 200 to 300 cells analyzed per each condition, and the background was subtracted automatically. Results were expressed as the difference between baseline and peak response ($\Delta F_{340/380}$), with data expressed as mean ±SEM (n = 5–7). Statistical analyses were performed by One-Way Analysis of Variance (ANOVA) and Newman-Keuls multiple comparisons test for data obtained in 0.25 or 2.5 mM Ca^{2+} using GraphPad Prism v6 (GraphPad Software Inc, La Jolla, CA, USA). A $p < 0.05$ was accepted as an indication of statistical significance.

3. Results

3.1. WIN55212-2 Increased $[Ca^{2+}]_i$ in N18TG2 Cells at Both Low eCa^{2+} and during a High-eCa^{2+} Stimulus

After resting at 0.25 mM extracellular Ca^{2+}, perfusion of N18TG2 cells with 0.25 mM eCa^{2+} increased $[Ca^{2+}]_i$ transiently by 11%. When eCa^{2+} was changed to 2.5 mM, $[Ca^{2+}]_i$ increased by 304% over basal ($\Delta F_{340/380}$ 0.11 ± 0.04 vs. 0.334 ± 0.06 $p < 0.05$, Figure 1A,B). We tested the Ca^{2+} mobilization response to the non-selective $CB_{1/2}R$ aminoalkylindole agonist WIN55212-2 at concentrations that have previously been demonstrated to stimulate Ca^{2+} mobilization in HEK293 cells [6]. In the presence of WIN55212-2 (5 μM), $[Ca^{2+}]_i$ increased by 700% over basal in 0.25 mM eCa^{2+} ($\Delta F_{340/380}$ 0.11 ± 0.04 vs. 0.84 ± 0.12 $p < 0.05$) and by 350% over basal in 2.5 mM eCa^{2+} ($\Delta F_{340/380}$ 0.334 ± 0.06 vs. 1.29 ± 0.13 $p < 0.05$, Figure 1B–D).

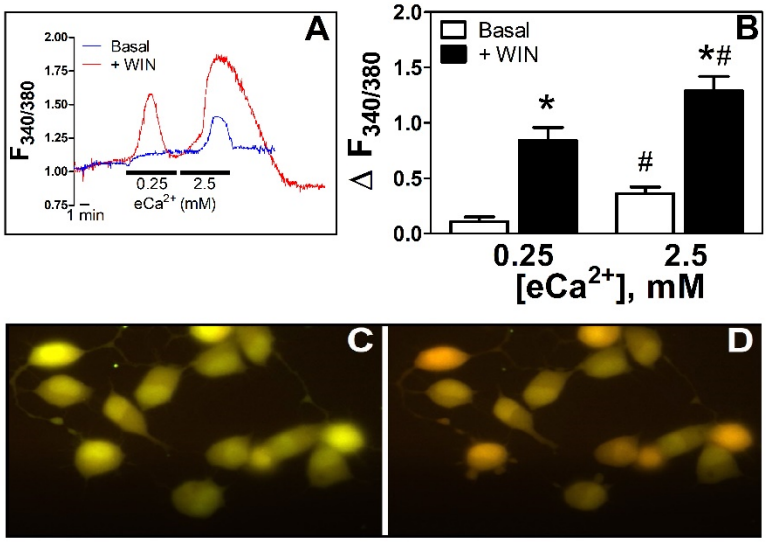

Figure 1. WIN55212-2 increases $[Ca^{2+}]_i$: effect of eCa^{2+}. (**A**). Time course of the changes in $F_{340/380}$ in N18TG2 cells incubated in 0.25 and 2.5 mM eCa^{2+} in basal conditions (blue line) or the presence of WIN55212-2 5 μM (red line). (**B**). Relative changes in $[Ca^{2+}]_i$ as $\Delta F_{340/380}$ in basal conditions (Basal, □, $n = 6$) or in the presence of WIN55212-2 5 μM (+WIN, ■, $n = 7$). * $p < 0.05$ vs. basal; # $p < 0.05$ vs. 0.25 mM eCa^{2+}. (**C**). Fluorescence image from a representative coverslip with N18TG2 cells observed under the imaging system used (see section Imaging in Materials and Methods) in eCa^{2+} 0.25 mM. 40× amplification. (**D**). Same cells as in C after 2 min treatment (approximately at peak response) with WIN55212-2 5 μM in eCa^{2+} 0.25 mM, 40x amplification. Changes in pseudo color from green to red represent the increase in emission after excitation at 340 nm and decrease in emission after excitation at 380 nm of Fura-2 upon binding to Ca^{2+}, the basis of the ratiometric ($F_{340/380}$) system for determinations of relative changes in $[Ca^{2+}]_i$ [17].

3.2. CaSR Mediates Increases in eCa^{2+}-Induced $[Ca^{2+}]_i$ in N18TG2 Cells

NPS2143 is a CaSR negative allosteric modulator that binds to the 7-transmembrane domain of the CaSR to inhibit the Ca^{2+} mobilization signaling pathway [21,22]. We used this calcilytic agent at concentrations previously shown to block increases in $[Ca^{2+}]_i$ promoted by activation of the Ca^{2+} receptor in HEK293 cells expressing the human Ca^{2+} receptor [18]. High eCa^{2+}-induced $[Ca^{2+}]_i$ increase was effectively antagonized with NPS2143 (3 μM) ($\Delta F_{340/380}$ 0.36 ± 0.06 vs. 0.16 ± 0.02, $p < 0.05$, 56% of reduction, Figure 2A), demonstrating the functional activity of the CaSR evident at supra-physiological eCa^{2+}. The WIN55212-2-induced elevations in $[Ca^{2+}]_i$ in low eCa^{2+} conditions were also attenuated by simultaneous perfusion with the NPS2143 (3 μM) ($\Delta F_{340/380}$ 0.84 ± 0.12 vs. 0.54 ± 0.05, $p < 0.05$, 36% of reduction, Figure 2B). Interestingly, in the presence of high eCa^{2+}, the WIN55212-2-induced $[Ca^{2+}]_i$ increase was not inhibited by NPS2143 ($\Delta F_{340/380}$ 1.29 ± 0.13 vs. 1.25 ± 0.14, $p > 0.05$). These findings might suggest that the WIN55212-2 can influence $[Ca^{2+}]_i$ under "basal" CaSR conditions, but the WIN55212-2 stimulus was not influenced by NPS2143-inhibited CaSR. An alternative interpretation is that WIN55212-2 provided a mechanism to protect the CaSR from inhibition by the negative allosteric modulator.

3.3. Aminoalkylindole-Specific Potentiation of the eCa^{2+}-Mediated Increase in $[Ca^{2+}]_i$

To check the selectivity of WIN55212-2-induced increase in $[Ca^{2+}]_i$ in neuronal cells, the non-classical cannabinoid full agonist, CP55940, and endocannabinoid partial agonist, methanandamide (Me-AEA) were used at concentrations previously shown to inhibit cAMP accumulation [23]. Both compounds at 5 μM failed to significantly increase $[Ca^{2+}]_i$ relative to basal values at either 0.25 mM ($\Delta F_{340/380}$ CP 0.29 ± 0.08, Me-AEA 0.05 ± 0.01, $p > 0.05$) or at 2.5 mM eCa^{2+} ($\Delta F_{340/380}$ CP 0.44 ± 0.03, Me-AEA 0.75 ± 0.17, $p > 0.05$, Figure 3). The responses to either CP55940 or Me-AEA on $[Ca^{2+}]_i$ at both levels of eCa^{2+} were significantly

lower than the response to WIN55212-2 ($p < 0.05$). These findings support the WIN55212-2 selectivity, implicating a non-CB_1 and non-CB_2 mechanism for this response.

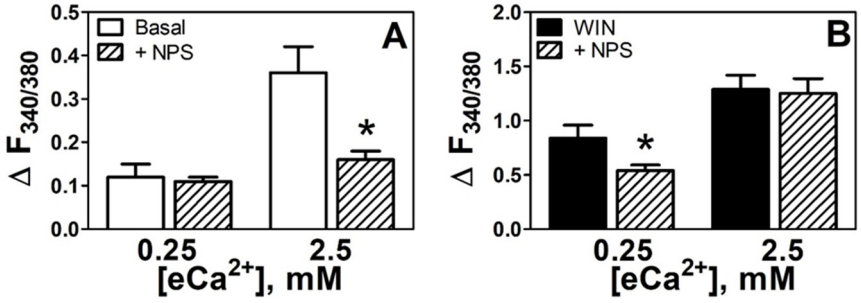

Figure 2. Increases in $[Ca^{2+}]_i$ depend on the CaSR. (**A**). Effects of the CaSR inhibitor NPS2314 3 μM (+NPS, diagonal stripes upward bars, $n = 7$) on $[Ca^{2+}]_i$ in basal conditions (open bars, $n = 6$). (**B**). Effects of the CaSR inhibitor NPS2314 3 μM (+NPS, diagonal stripes upward bars, $n = 7$) on $[Ca^{2+}]_i$ in the presence of WIN55212-2 5 μM (black bars, $n = 7$). * $p < 0.05$ vs. basal or WIN55212-2 at the corresponding eCa^{2+}.

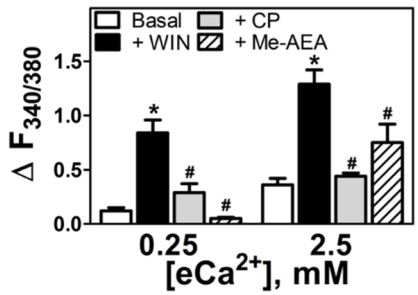

Figure 3. Aminoalkylindole-specific increase in $[Ca^{2+}]_i$ in N18TG2 cells. Increases in $[Ca^{2+}]_i$ in conditions of low (0.25 mM) and high (2.5 mM) eCa^{2+} in the presence of WIN55212-2 5μM (+WIN, $n = 7$), the bicyclic mimetic of THC CP55940 5 μM (+CP, $n = 4$), or the arachidonoylethanolamine analog meth-anandamide 5 μM (+Me-AEA, $n = 5$) * $p < 0.05$ vs. basal; # $p < 0.05$ vs. WIN55212-2.

3.4. WIN55212-2-Stimulated Increases in $[Ca^{2+}]_i$ Require Operational Store Operated Calcium Entry (SOCE)

Ca^{2+} mobilization by GPCR-mediated production of inositol triphosphate (IP_3) promotes Ca^{2+} release from ER stores, which requires continuous repletion via store operated Ca^{2+} entry (SOCE) mechanisms [24,25]. The most effective SOCE mechanism is based upon the ER $[Ca^{2+}]$ sensor stromal interacting molecule (STIM) and its association and activation of Ca^{2+} release-activated Ca^{2+} channels (CRAC) comprised of Orai1, Orai2, and Orai3 proteins. Cells also utilize non-selective cation channels as store-operated channels (SOCs), comprised of both Orai1 and transient receptor potential canonical channel 1 (TRPC1) channel subunits. Current reviews describe these processes in detail [25–28].

To evaluate the role of SOCE in WIN55212-2-stimulated increases in $[Ca^{2+}]_i$, we employed the Orai1 inhibitor N-propargyl-nitrendipine (MRS1845), which has a reported $IC_{50} = 1.7$ μM to block capacitative Ca^{2+} influx in HL60 cells [19], and also inhibits the ER Ca^{2+} replacement via TRPC1 at higher concentrations [29]. The WIN55212-2-stimulated increases in $[Ca^{2+}]_i$, in both eCa^{2+} conditions (0.25 mM or 2.5 mM Ca^{2+}) were attenuated by incubation with MRS1845 (10 μM) ($\Delta F_{340/380}$ MRS 0.33 ± 0.07, $n = 4$, 61% reduction at 0.25 mM; $\Delta F_{340/380}$ MRS 0.7 ± 0.14, $n = 7$, 46% reduction at 2.5 mM, Figure 4, $p < 0.05$). These results are consistent with a requirement for continuous refilling of the intracellular Ca^{2+} stores in the ER as the source of the mobilized Ca^{2+}.

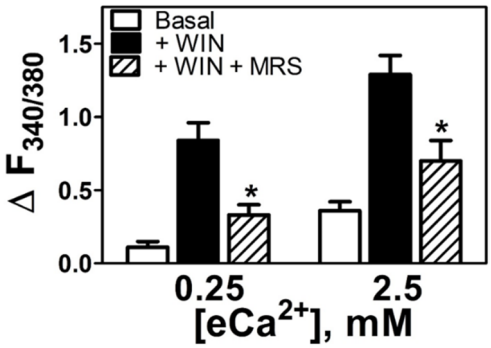

Figure 4. WIN55212-2-mediated increase in $[Ca^{2+}]_i$ is dependent on store operated calcium entry. Increases in $[Ca^{2+}]_i$ in low (0.25 mM) and high (2.5 mM) eCa^{2+} in basal conditions (Basal, $n = 6$), in the presence of WIN55212-2 (5 μM, $n = 7$) (+WIN), and in the presence of WIN55212-2 plus MRS1845 (+WIN + MRS, $n = 4$ or $n = 7$) (10 μM). * $p < 0.05$ vs. +WIN.

3.5. WIN55212-2-Dependent Increases in $[Ca^{2+}]_i$ Are Mediated by either CB_1R or a nonCB$_1$/CB$_2$ Receptor as a Function of the eCa^{2+} Stimulus

The N18TG2 neuronal cell expresses CB_1R but fails to express CB_2R [30–32], and thus, cellular signaling via cannabinoid receptors is expected to be inhibited by a CB_1R competitive antagonist/inverse agonist such as SR141716 in this model [33]. Several non-CB_1, non-CB_2 GPCRs have been promoted as "Cannabinoid Related" receptors based on their ability to be orthosterically stimulated/inhibited or allosterically modified by phytocannabinoid or endocannabinoid-like compounds (see [34–36] for review). The cannabinoid related GPCRs GPR18 and GPR55 both signal through Ca^{2+} mobilization, and both interact with endocannabinoid-like N-arachidonoylglycine and N-arachidonoylserine, phytocannabinoid CBD, and CBD analogs abnormal-cannabidiol (abn-CBD), O-1602 and O-1918 [37]. For this reason, we chose to test O-1918 for its potential as an inhibitor of Ca^{2+} mobilization in these studies, and we selected a concentration of O-1918 (10 μM) that has been shown to block cannabinoid-dependent effects that are independent of CB_1R or CB_2R [20]. The WIN55212-2-induced increase in $[Ca^{2+}]_i$ in 0.25 mM eCa^{2+} was partially blocked by simultaneous perfusion with the CB_1R antagonist SR141716 (1 μM) ($\Delta F_{340/380}$ SR 0.45 ± 0.07, $n = 6$, $p < 0.05$, 46% reduction). Under conditions of 2.5 mM eCa^{2+}, SR141716 does not affect the WIN55212-2-dependent increase in $[Ca^{2+}]_i$ ($\Delta F_{340/380}$ SR 1.57 ± 0.11, $n = 6$, $p > 0.05$). Simultaneous perfusion with the nonCB$_1$/CB$_2$ receptor antagonist O-1918 (10 μM), attenuated WIN55212-2-promoted increases in $[Ca^{2+}]_i$ at both eCa^{2+} levels ($\Delta F_{340/380}$ O-1918 0.28 ± 0.07, $n = 5$, $p < 0.05$, 67% reduction at 0.25 mM; $\Delta F_{340/380}$ O-1918 0.72 ± 0.05, $n = 5$, $p < 0.05$, 44% reduction at 2.5 mM, Figure 5B). These findings implicate the role of the CB_1R in WIN55212-2-promoted $[Ca^{2+}]_i$ increases in the absence of a CaSR stimulus. On the other hand, a prominent influence of a nonCB$_1$/CB$_2$ stimulus appears under conditions of activation of CaSR by supra-physiological eCa^{2+}.

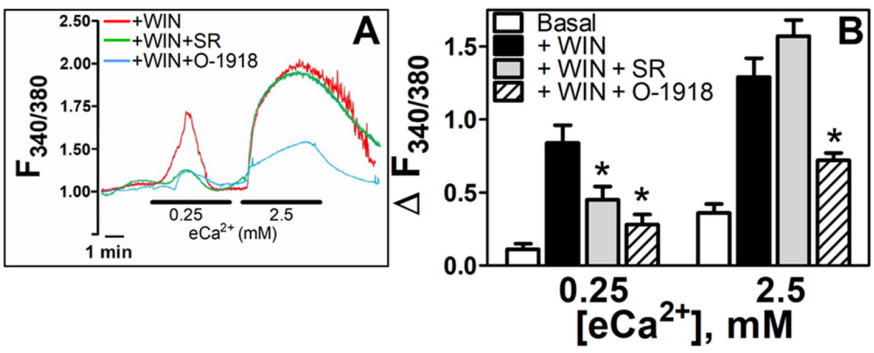

Figure 5. WIN55212-2-mediated increase in $[Ca^{2+}]_i$ is dependent on the CB_1 receptor and on nonCB$_1$/CB$_2$ receptors. (**A**). Time course of the changes in $F_{340/380}$ in N18TG2 cells incubated in 0.25

and 2.5 mM eCa^{2+} in the presence of WIN55212-2 5 μM (+WIN, red line), SR141716 1 μM (+WIN + SR, green line) and WIN55212-2 plus O-1918 10 μM (+WIN + O-1918, light blue line). (**B**). Increases in $[Ca^{2+}]_i$ in low (0.25 mM) and high (2.5 mM) eCa^{2+} in basal conditions (Basal, open bars, $n = 6$), in the presence of WIN55212-2 (5 μM) (+WIN, $n = 7$), in the presence of WIN55212-2 plus SR141716 (1 μM) (+WIN + SR, $n = 7$), and in the presence of WIN55212-2 plus O-1918 (10 μM) (+WIN + O-1918, $n = 5$). * $p < 0.05$ vs. +WIN.

4. Discussion

The aminoalkylindole WIN55212-2 can modulate $[Ca^{2+}]_i$ in neuroblastoma cells via at least two mechanisms. At low eCa^{2+}, the WIN55212-2 induced potentiation of $[Ca^{2+}]_i$ partially depends on CB_1R defined by its sensitivity to inhibition by SR141716. At supra-physiologic eCa^{2+}, which activates the CaSR, the effect of WIN55212-2 is CB_1R-independent. At both eCa^{2+} levels, the release of Ca^{2+} is from intracellular stores filled by store-operated Ca^{2+} channels.

We observed that the effect of WIN55212-2 on $[Ca^{2+}]_i$ depends on the eCa^{2+} level. At low eCa^{2+}, WIN55212-2 increases $[Ca^{2+}]_i$ acting via CB_1R and nonCB_1/CB_2 receptors, probably acting on different intracellular transduction pathways. Actions of CB_1R through pertussis toxin-sensitive $G_{i/o}$ proteins leading to inhibition of cAMP production were first demonstrated in N18TG2 neuroblastoma cells [38]. CB_1R acting through $G\alpha_i$ could serve a modulatory role, as has also been proposed for $G\alpha_q$ signaling, in mediating $[Ca^{2+}]_i$ increases in these cells [12]. The requirement for $G\alpha_q$ on increasing $[Ca^{2+}]_i$ after CB_1R activation was demonstrated in HEK293 cells and hippocampal neurons [6].

Our results of an eCa^{2+}-dependent elevation in $[Ca^{2+}]_i$ confirm a role for CaSR in the modulation of $[Ca^{2+}]_i$ in N18TG2 neuroblastoma cells, as previously demonstrated [12]. The effects of WIN55212-2 we report at low and high eCa^{2+} were attenuated by SOCE blockade, suggesting that WIN55212-2 promotes the release of Ca^{2+} from intracellular stores. The main transduction pathway associated with this particular increase in $[Ca^{2+}]_i$ is described as dependent on $G\alpha_q$, phospholipase C (PLC) activation, synthesis of diacylglycerol (DAG), and inositol triphosphate (IP_3) with further activation of ER IP_3 receptors promoting Ca^{2+} release [11]. PLC can be activated by either $G\alpha_q$ or $G_{i/o}$ $\beta\gamma$ subunits, with these two effectors interacting with distinct regions of PLCs; $G\alpha_q$ binds to the C-terminal and $G\beta\gamma$ binds to the catalytic domain [39]. $G\alpha_q$ and $G_{i/o}$ $\beta\gamma$ can cooperate synergistically, increasing $[Ca^{2+}]_i$ after GPCR activation [40]. Recently a role for $G_{i/o}$ $\beta\gamma$ subunits as modulators of $G\alpha_q$ activation of PLC, forming a $G\alpha_q$-PLC-$G_{i/o}$ $\beta\gamma$ complex, and depending on the affinity for the plasma membrane of the γ subunits has been proposed [41]. Regarding potential interactions between CaSR- and cannabinoid-dependent pathways modulating $[Ca^{2+}]_i$, it is conceivable that WIN55212–2-dependent CB_1R activation increases $[Ca^{2+}]_i$ by augmenting CaSR-$G\alpha_q$-dependent activation of PLC with the participation of CB_1R-mediated $G_{i/o}$ $\beta\gamma$ release in neuroblastoma cells.

Two conundrums remain. One is that only the WIN55212-2 but no other cannabinoid or endocannabinoid agonist family representatives could stimulate the Ca^{2+} mobilization. The other is that we observed a role for CaSR in WIN55212-2/CB_1R-dependent increases in $[Ca^{2+}]_i$ at low eCa^{2+} and not at high eCa^{2+}. These results suggest the possibility of different $G\alpha_q$-PLC-$G_{i/o}$ $\beta\gamma$ complexes being formed depending on eCa^{2+} and the cellular proximity of the receptors and the G proteins to which they are pre-coupled (Figure 6). For example, our earlier studies demonstrated that WIN55212-2 behaves as an agonist for all three Gi subtypes, whereas the THC analog desacetyllevonantradol behaves as an agonist for Gi_1 and Gi_2 but an inverse agonist for Gi_3; and methanandamide behaves as an agonist at Gi_3 but an inverse agonist for Gi_1 and Gi_2 [42].

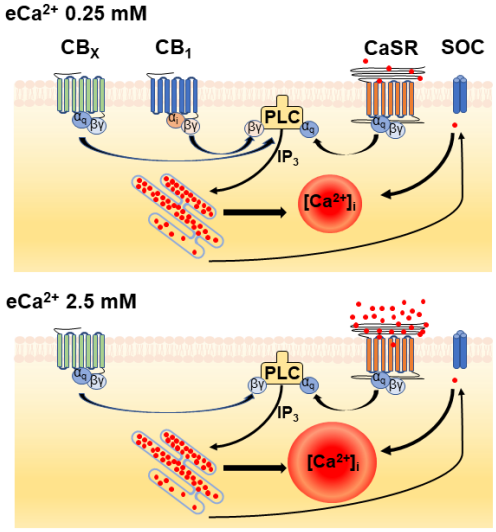

Figure 6. Diagram illustrating potential functional interactions between cannabinoid receptors and CaSR in neuroblastoma cells in different levels of eCa^{2+}. The formation of different $G\alpha_q$-PLC-$G_{i/o}$ $\beta\gamma$ complexes depending on eCa^{2+} and cellular proximity of the GPCRs involved is proposed. CB_1: CB_1 receptor. CBx: putative 'WIN55212-2' receptor. CaSR: calcium sensing receptor. SOC: store operated calcium channels.

In the current study, we also showed a role for nonCB_1/CB_2 receptors in controlling $[Ca^{2+}]_i$. Among the candidates for these receptors is GPR55, which can be activated by CB_1R antagonist/inverse agonist AM251 (but not SR141716), and the lysophospholipid, lysophosphatidylinositol (LPI). GPR55 utilizes $G\alpha_q$ or $G\alpha_{12/13}$ for signal transduction [43] and can promote Ca^{2+} mobilization and mitogen-activated protein kinase (MAPK) phosphorylation [44]. GPR55 modulates neurotransmitter release through modulation of neuronal $[Ca^{2+}]_i$ [45]. Activation of GPR55 in dorsal root ganglia neurons by various cannabinoids, including Δ^9-THC and methanandamide, increases $[Ca^{2+}]_i$ through a mechanism involving $G\alpha_q$, PLC, and IP_3 receptors [7].

Our results with O-1918, could implicate a role for GPR55. However, since WIN55212-2 fails to activate $[Ca^{2+}]_i$ by GPR55 [7] the effects of O-1918 on WIN55212-2-dependent responses we observed may be explained by the CB_1-induced activation of phospholipase A and synthesis of LPI, which in turn would activate GPR55. This possibility has been suggested to explain the observed actions of CB_1R antagonists/inverse agonists on GPR55-mediated actions [46].

Another possibility for the nonCB_1/CB_2 receptor is GPR18, which originally was characterized by its activation by abnormal cannabidiol (abn-CBD) and inhibition by CBD and O-1918, but now de-orphanized as a GPCR activated by the endogenous anandamide metabolite, N-arachidonoyl-glycine (see review for original references [34,37]). In exogenous expression models, GPR18 responds to N-arachidoyl-glycine by Ca^{2+} mobilization [47]. GPR18 also responds to the inflammation pro-resolving polyunsaturated, hydroxylated 22-C lipid, resolvin D2 (RvD2). In monocytes, macrophages, microglia, and BV2 microglia, and polymorphonuclear neutrophils, GPR18 couples to $G_{i/o}$, $G_{q/11}$, and G_s to signal by increasing cAMP and protein kinase A (PKA), and phosphorylation of signal transducer and activator of transcription 3 (STAT3) (for review and original references, see [37,48]). However, WIN55212-2 has not been demonstrated to elicit any signaling responses by GPR18 (see summary tables and text for original references [37,48]).

A final possibility is that WIN55212-2 might activate a putative Alkyl Indole receptor as described for microglia and astrocyte cellular signaling in response to WIN55212-2 and analogs [49,50]. Such receptors may be those identified as [^{3}H]WIN55212-2 binding sites in neuroblastoma-glioma hybrid NG108-15 cells [51]. The potential for a specific WIN55212-2 "receptor" in brain membranes was suggested by studies of the C57Bl/6

CB_1 knock-out mouse which showed that anandamide and WIN55212-2 could promote G protein activation ([^{35}S]GTPγS binding) [52]. The properties of a putative Alkyl Indole receptor have yet to be fully characterized.

In summary, the aminoalkylindole WIN55212-2 can modulate $[Ca^{2+}]_i$ in N18TG2 cells via CB_1R-dependent and independent mechanisms. We observed functional interactions between the CB_1R and CaSR activation in regulating $[Ca^{2+}]_i$, and these interactions are dependent on eCa^{2+} with the participation of nonCB_1/CB_2 receptors in neuroblastoma cells. Future studies should address the effects of LPI, abn-CBD, CBD and RvD2 to determine the role of GPR55 and GPR18 on Ca^{2+} mobilization influenced by the CaSR. Additionally, studies should address whether aminoalkylindole analogs that act on the putative WIN55212-2 Alkyl Indole "receptor" are involved in the regulation of $[Ca^{2+}]_i$ by CaSR. Details regarding the mechanism by which the CaSR interfaces with Class A GPCRs to regulate intracellular calcium stores could be analyzed using thapsigargin-dependent control of $[Ca^{2+}]_i$ [6] and other current methods of structural and functional analysis.

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References

1. Felder, C.C.; Veluz, J.S.; Williams, H.L.; Briley, E.M.; Matsuda, L.A. Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. *Mol. Pharmacol.* **1992**, *42*, 838–845. [PubMed]
2. Carney, S.T.; Lloyd, M.L.; MacKinnon, S.E.; Newton, D.C.; Jones, J.D.; Howlett, A.C.; Norford, D.C. Cannabinoid regulation of nitric oxide synthase I (nNOS) in neuronal cells. *J. Neuroimmune Pharmacol.* **2009**, *4*, 338–349. [CrossRef] [PubMed]
3. Felder, C.C.; Joyce, K.E.; Briley, E.M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A.L.; Mitchell, R.L. Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. *Mol. Pharmacol.* **1995**, *48*, 443–450. [PubMed]
4. Sugiura, T.; Kondo, S.; Kishimoto, S.; Miyashita, T.; Nakane, S.; Kodaka, T.; Suhara, Y.; Takayama, H.; Waku, K. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB_2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *J. Biol. Chem.* **2000**, *275*, 605–612. [CrossRef]
5. Sugiura, T.; Kodaka, T.; Kondo, S.; Tonegawa, T.; Nakane, S.; Kishimoto, S.; Yamashita, A.; Waku, K. 2-Arachidonoylglycerol, a putative endogenous cannabinoid receptor ligand, induces rapid, transient elevation of intracellular free Ca^{2+} in neuroblastoma × glioma hybrid NG108-15 cells. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 58–64. [CrossRef]
6. Lauckner, J.E.; Hille, B.; Mackie, K. The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB_1 receptor coupling to Gq/11 G proteins. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 19144–19149. [CrossRef]
7. Lauckner, J.E.; Jensen, J.B.; Chen, H.Y.; Lu, H.C.; Hille, B.; Mackie, K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2699–2704. [CrossRef]
8. Berridge, M.J. The inositol trisphosphate/calcium signaling pathway in health and disease. *Physiol. Rev.* **2016**, *96*, 1261–1296. [CrossRef]

9. Gerbino, A.; Colella, M. The different facets of extracellular calcium sensors: Old and new concepts in calcium-sensing receptor signalling and pharmacology. *Int. J. Mol. Sci.* **2018**, *19*, 999. [CrossRef]
10. Neves, S.R.; Ram, P.T.; Iyengar, R. G protein pathways. *Science* **2002**, *296*, 1636–1639. [CrossRef]
11. Gorkhali, R.; Tian, L.; Dong, B.; Bagchi, P.; Deng, X.; Pawar, S.; Duong, D.; Fang, N.; Seyfried, N.; Yang, J. Extracellular calcium alters calcium-sensing receptor network integrating intracellular calcium-signaling and related key pathway. *Sci. Rep.* **2021**, *11*, 20576. [CrossRef] [PubMed]
12. Sesay, J.S.; Gyapong, R.N.; Najafi, L.T.; Kabler, S.L.; Diz, D.I.; Howlett, A.C.; Awumey, E.M. Gαi/o-dependent Ca^{2+} mobilization and Gαq-dependent PKCα regulation of Ca^{2+}-sensing receptor-mediated responses in N18TG2 neuroblastoma cells. *Neurochem. Int.* **2015**, *90*, 142–151. [CrossRef] [PubMed]
13. Awumey, E.M.; Howlett, A.C.; Putney, J.W.; Diz, D.I.; Bukoski, R.D. Ca^{2+} mobilization through dorsal root ganglion Ca^{2+}-sensing receptor stably expressed in HEK293 cells. *Am. J. Physiol. Cell Physiol.* **2007**, *292*, C1895–C1905. [CrossRef] [PubMed]
14. Haddad, M. The impact of CB1 receptor on nuclear receptors in skeletal muscle cells. *Pathophysiology* **2021**, *28*, 457–470. [CrossRef] [PubMed]
15. Boczek, T.; Zylinska, L. Receptor-dependent and independent regulation of voltage-gated Ca^{2+} channels and Ca^{2+}-permeable channels by endocannabinoids in the brain. *Int. J. Mol. Sci.* **2021**, *22*, 8168. [CrossRef]
16. Eldeeb, K.; Leone-Kabler, S.; Howlett, A.C. Mouse neuroblastoma CB_1 cannabinoid receptor-stimulated [^{35}S]GTPγS binding: Total and antibody-targeted Gα protein-specific scintillation proximity assays. *Methods Enzymol.* **2017**, *593*, 1–21. [CrossRef]
17. Grynkiewicz, G.; Poenie, M.; Tsien, R.Y. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **1985**, *260*, 3440–3450. [CrossRef]
18. Nemeth, E.F.; Delmar, E.G.; Heaton, W.L.; Miller, M.A.; Lambert, L.D.; Conklin, R.L.; Gowen, M.; Gleason, J.G.; Bhatnagar, P.K.; Fox, J. Calcilytic compounds: Potent and selective Ca^{2+} receptor antagonists that stimulate secretion of parathyroid hormone. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 323–331.
19. Harper, J.L.; Camerini-Otero, C.S.; Li, A.H.; Kim, S.A.; Jacobson, K.A.; Daly, J.W. Dihydropyridines as inhibitors of capacitative calcium entry in leukemic HL-60 cells. *Biochem. Pharmacol.* **2003**, *65*, 329–338. [CrossRef]
20. Hoi, P.M.; Hiley, C.R. Vasorelaxant effects of oleamide in rat small mesenteric artery indicate action at a novel cannabinoid receptor. *Br. J. Pharmacol.* **2006**, *147*, 560–568. [CrossRef]
21. Davey, A.E.; Leach, K.; Valant, C.; Conigrave, A.D.; Sexton, P.M.; Christopoulos, A. Positive and negative allosteric modulators promote biased signaling at the calcium-sensing receptor. *Endocrinology* **2012**, *153*, 1232–1241. [CrossRef] [PubMed]
22. Wen, T.; Wang, Z.; Chen, X.; Ren, Y.; Lu, X.; Xing, Y.; Lu, J.; Chang, S.; Zhang, X.; Shen, Y.; et al. Structural basis for activation and allosteric modulation of full-length calcium-sensing receptor. *Sci. Adv.* **2021**, *7*, eabg1483. [CrossRef]
23. Khajehali, E.; Malone, D.T.; Glass, M.; Sexton, P.M.; Christopoulos, A.; Leach, K. Biased agonism and biased allosteric modulation at the CB_1 cannabinoid receptor. *Mol. Pharmacol.* **2015**, *88*, 368–379. [CrossRef] [PubMed]
24. Lewis, R.S. Store-Operated Calcium Channels: From function to structure and back again. *Cold Spring Harb. Perspect. Biol.* **2020**, *12*, a035055. [CrossRef] [PubMed]
25. Prakriya, M.; Lewis, R.S. Store-Operated Calcium Channels. *Physiol. Rev.* **2015**, *95*, 1383–1436. [CrossRef] [PubMed]
26. Bird, G.S.; Putney, J.W., Jr. Pharmacology of store operated calcium entry channels. In *Calcium Entry Channels in Non-Excitable Cells*; Kozak, J.A., Putney, J.W., Eds.; CRC Press/Taylor & Francis Group, LLC: Boca Raton, FL, USA, 2018; pp. 311–324.
27. Putney, J.W. Forms and functions of store-operated calcium entry mediators, STIM and Orai. *Adv. Biol. Regul.* **2018**, *68*, 88–96. [CrossRef] [PubMed]
28. Lopez, J.J.; Albarran, L.; Gómez, L.J.; Smani, T.; Salido, G.M.; Rosado, J.A. Molecular modulators of store-operated calcium entry. *Biochim. Biophys. Acta* **2016**, *1863*, 2037–2043. [CrossRef]
29. Bomben, V.C.; Sontheimer, H. Disruption of transient receptor potential canonical channel 1 causes incomplete cytokinesis and slows the growth of human malignant gliomas. *Glia* **2010**, *58*, 1145–1156. [CrossRef]
30. Eldeeb, K.; Ganjiwale, A.D.; Chandrashekaran, I.R.; Padgett, L.W.; Burgess, J.; Howlett, A.C.; Cowsik, S.M. CB1 cannabinoid receptor-phosphorylated fourth intracellular loop structure-function relationships. *Pept. Sci.* **2019**, *111*, e24104. [CrossRef]
31. Mukhopadhyay, S.; Das, S.; Williams, E.A.; Moore, D.; Jones, J.D.; Zahm, D.S.; Ndengele, M.M.; Lechner, A.J.; Howlett, A.C. Lipopolysaccharide and cyclic AMP regulation of CB_2 cannabinoid receptor levels in rat brain and mouse RAW 264.7 macrophages. *J. Neuroimmunol.* **2006**, *181*, 82–92. [CrossRef]
32. Jones, J.D.; Carney, S.T.; Vrana, K.E.; Norford, D.C.; Howlett, A.C. Cannabinoid receptor-mediated translocation of NO-sensitive guanylyl cyclase and production of cyclic GMP in neuronal cells. *Neuropharmacology* **2008**, *54*, 23–30. [CrossRef] [PubMed]
33. Howlett, A.C.; Abood, M.E. CB_1 and CB_2 Receptor Pharmacology. *Adv. Pharmacol.* **2017**, *80*, 169–206. [CrossRef] [PubMed]
34. Morales, P.; Reggio, P.H. An update on non-CB_1, non-CB_2 cannabinoid related G-Protein-coupled receptors. *Cannabis Cannabinoid Res.* **2017**, *2*, 265–273. [CrossRef] [PubMed]
35. Ye, L.; Cao, Z.; Wang, W.; Zhou, N. New insights in cannabinoid receptor structure and signaling. *Curr. Mol. Pharmacol.* **2019**, *12*, 239–248. [CrossRef]
36. Pertwee, R.G.; Howlett, A.C.; Abood, M.E.; Alexander, S.P.; Di Marzo, V.; Elphick, M.R.; Greasley, P.J.; Hansen, H.S.; Kunos, G.; Mackie, K.; et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB_1 and CB_2. *Pharmacol. Rev.* **2010**, *62*, 588–631. [CrossRef]

37. Morales, P.; Lago-Fernandez, A.; Hurst, D.P.; Sotudeh, N.; Brailoiu, E.; Reggio, P.H.; Abood, M.E.; Jagerovic, N. Therapeutic exploitation of GPR18: Beyond the cannabinoids? *J. Med. Chem.* **2020**, *63*, 14216–14227. [CrossRef]
38. Howlett, A.C.; Qualy, J.M.; Khachatrian, L.L. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol. Pharmacol.* **1986**, *29*, 307–313.
39. Rhee, S.G. Regulation of phosphoinositide-specific phospholipase C. *Annu. Rev. Biochem.* **2001**, *70*, 281–312. [CrossRef]
40. Rebres, R.A.; Roach, T.I.; Fraser, I.D.; Philip, F.; Moon, C.; Lin, K.M.; Liu, J.; Santat, L.; Cheadle, L.; Ross, E.M.; et al. Synergistic Ca^{2+} responses by Gαi- and Gαq-coupled G-protein-coupled receptors require a single PLCβ isoform that is sensitive to both Gβγ and Gαq. *J. Biol. Chem.* **2011**, *286*, 942–951. [CrossRef]
41. Kankanamge, D.; Ubeysinghe, S.; Tennakoon, M.; Pantula, P.D.; Mitra, K.; Giri, L.; Karunarathne, A. Dissociation of the G protein βγ from the Gq-PLCβ complex partially attenuates PIP_2 hydrolysis. *J. Biol. Chem.* **2021**, *296*, 100702. [CrossRef]
42. Mukhopadhyay, S.; Howlett, A.C. Chemically distinct ligands promote differential CB_1 cannabinoid receptor-Gi protein interactions. *Mol. Pharmacol.* **2005**, *67*, 2016–2024. [CrossRef] [PubMed]
43. Sharir, H.; Abood, M.E. Pharmacological characterization of GPR55, a putative cannabinoid receptor. *Pharmacol. Ther.* **2010**, *126*, 301–313. [CrossRef] [PubMed]
44. Console-Bram, L.; Ciuciu, S.M.; Zhao, P.; Zipkin, R.E.; Brailoiu, E.; Abood, M.E. N-arachidonoyl glycine, another endogenous agonist of GPR55. *Biochem. Biophys. Res. Commun.* **2017**, *490*, 1389–1393. [CrossRef]
45. Sylantyev, S.; Jensen, T.P.; Ross, R.A.; Rusakov, D.A. Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5193–5198. [CrossRef]
46. De Petrocellis, L.; Di Marzo, V. Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: Focus on G-protein-coupled receptors and transient receptor potential channels. *J. Neuroimmune Pharmacol.* **2010**, *5*, 103–121. [CrossRef] [PubMed]
47. Console-Bram, L.; Brailoiu, E.; Brailoiu, G.C.; Sharir, H.; Abood, M.E. Activation of GPR18 by cannabinoid compounds: A tale of biased agonism. *Br. J. Pharmacol.* **2014**, *171*, 3908–3917. [CrossRef]
48. Park, J.; Langmead, C.J.; Riddy, D.M. New advances in targeting the resolution of inflammation: Implications for specialized pro-resolving mediator GPCR drug discovery. *ACS Pharmacol. Transl. Sci.* **2020**, *3*, 88–106. [CrossRef]
49. Fung, S.; Cherry, A.E.; Xu, C.; Stella, N. Alkylindole-sensitive receptors modulate microglial cell migration and proliferation. *Glia* **2015**, *63*, 1797–1808. [CrossRef]
50. Stella, N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* **2010**, *58*, 1017–1030. [CrossRef]
51. Stark, S.; Pacheco, M.A.; Childers, S.R. Binding of aminoalkylindoles to noncannabinoid binding sites in NG108-15 cells. *Cell. Mol. Neurobiol.* **1997**, *17*, 483–493. [CrossRef]
52. Breivogel, C.S.; Griffin, G.; Di Marzo, V.; Martin, B.R. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol.* **2001**, *60*, 155–163. [CrossRef] [PubMed]