



# Differential effects of endocannabinoid signaling on spontaneous and evoked GABA release from retinal amacrine cells.

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### Introduction:

In the present study, we have investigated the role of endocannabinoids in synaptic transmission in the retina.

We have examined both asynchronous evoked and "spontaneous" transmission at autapses using cultured chick amacrine cells. We show that the cannabinoid receptor type 1 (CB<sub>1</sub>R) agonist, Win-55212,2 inhibits asynchronous evoked transmission via a non-CB<sub>1</sub>R pathway and activates "spontaneous transmission" via CB<sub>1</sub>R. The increase in "spontaneous" transmitter release is observed in amacrine cells having a low basal rate of transmission. We show that this phenomenon is presynaptic in origin and requires G<sub>i/o</sub> activation.

### Methods:

We performed immunocytochemistry using primary antibodies against CB<sub>1</sub>R and SV2 on cultured amacrine cells (Embryonic Equivalent days 14 through 16), that were fixed in paraformaldehyde. Subsequently, we stained the cells using suitable fluorescent secondary antibodies (Alexa dye conjugated antibodies, Molecular Probes, Inc.) and viewed them using confocal microscopy. Images were analyzed and merged using Matlab 6.0 and Photoshop 6.

Patch clamp experiments were performed in the whole cell mode using normal external solution containing TTX and a Cs<sup>+</sup> based internal solution (E<sub>Cl</sub> = 0 mV). Cells were usually held at -70 mV. Ca<sup>2+</sup> currents were recorded during a ramp protocol from -70 to +40 mV.

To measure asynchronous evoked transmitter release, we first depolarized cells to 0 mV for 1 s and then hyperpolarized them to -80 mV for 10 s. Minis recorded during the 10 s hyperpolarization step were counted and the frequency reported either as the mean mini frequency or the frequency normalized to control.

### Immunocytochemistry

Antibody staining experiments for both CB<sub>1</sub>R and SV2 reveal a punctate overlapping pattern along the dendrites of cultured amacrine cells. This suggests that CB<sub>1</sub>R are largely localized at autaptic and synaptic sites.

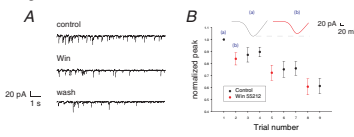
Fig. 1



### Asynchronous evoked transmitter release

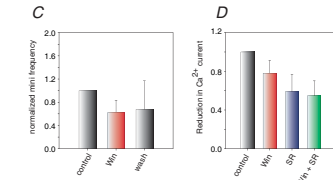
Win-55212,2 (Win) (5 - 10 μM) reduced the frequency of evoked minis (Fig. 2 A,C, n = 11, 38 ± 21% reduction). This is probably due to inhibition of Ca<sup>2+</sup> channels as confirmed by ramp protocols (representative experiment shown in Fig 2B, n = 9).

Fig 2



However, SR-141716 (SR) (5 μM), a specific CB<sub>1</sub>R antagonist, also inhibited Ca<sup>2+</sup> currents (n = 6). When SR was coapplied with Win, it further inhibited Ca<sup>2+</sup> currents (Fig. 2D, n = 9).

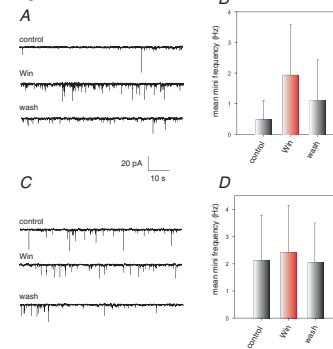
This suggests that Win-induced Ca<sup>2+</sup> channel inhibition is non-CB<sub>1</sub>R dependent and is mediated by an unknown receptor or process.



### Spontaneous transmitter release

Win (10 μM) increased the frequency of spontaneously occurring minis in a subpopulation of amacrine cells. The boost in mini frequency upon Win application was more significant in cells with low spontaneous transmission rate (~ 0.5 Hz, n = 7, Fig. 3 A,B) than in cells with a higher resting transmission rate (~ 2 Hz, n = 4, Fig. 3 C,D).

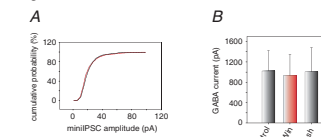
Fig. 3



### Increase in spontaneous transmission is mediated presynaptically

Win application did not affect either the amplitude distribution of spontaneous minis (Fig. 4A, Kolmogorov-Smirnov test) or GABA<sub>A</sub> current, induced by a 5 s application of 150 μM GABA (Fig. 4B, n = 12). Hence, we conclude that the effect is presynaptic.

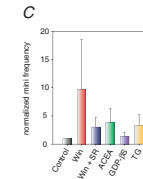
Fig 4.



### Win effect is G protein mediated and ER Ca<sup>2+</sup> Store-independent

Win-induced increase in spontaneous transmission was blocked when SR (5 μM) was coapplied (n = 6) or when GDP-βS (5 mg/ml) was included in the recording pipet (n = 5), suggesting a CB<sub>1</sub>R mediated and G protein coupled process (Fig. 4C). Treating cells with 1.8 μM thapsigargin for 1 hr did not affect the Win-induced increase in spontaneous transmission (Fig. 4C, n = 6). Also, application of Win did not increase cytoplasmic Ca<sup>2+</sup> (data not shown), suggesting that increased transmission is ER Ca<sup>2+</sup> store-independent.

ACEA, another specific CB<sub>1</sub>R agonist, also increased mini frequency (concentration = 30 μM, n = 12).



### Increasing cAMP blocks the Win effect

Increasing cytoplasmic cAMP either by treating with IBMX (a phosphodiesterase inhibitor, Fig. 5A, n = 5, 100 μM for 15min) or by co-applying 8-Br-cAMP (Fig. 5B, n = 6, 100 μM), inhibited the Win effect of increasing spontaneous minis.

Fig. 5

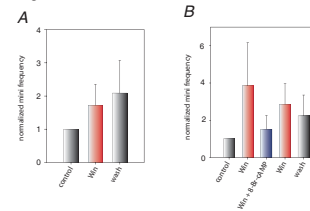
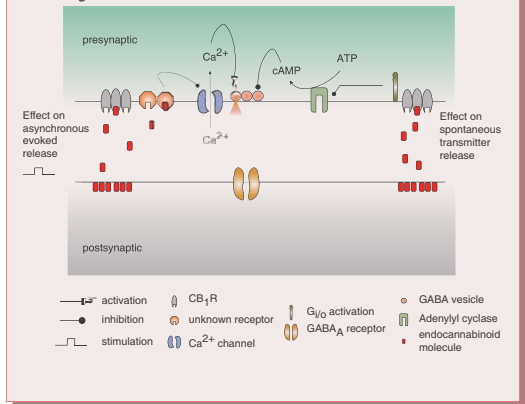


Fig. 6



### Conclusions:

1. Win-55212,2 inhibits Ca<sup>2+</sup> channels and asynchronous evoked neurotransmission through a non-CB<sub>1</sub>R mechanism.
2. Win-55212,2 increases spontaneous transmission in the population of cells with a low resting rate of observed minis (~0.5 Hz).
3. The increase in spontaneous transmission mediated is presynaptically through CB<sub>1</sub>R.
4. Increased spontaneous transmission is not ER Ca<sup>2+</sup> store-dependent, but is mediated by a G protein coupled process.
5. We conclude that increasing cytoplasmic cAMP inhibits spontaneous transmission and CB<sub>1</sub>R activation increases spontaneous transmission by reducing cAMP production through G<sub>i/o</sub> activation.

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