Beyond medicinal cannabis: from Pot to PAMs and NAMs

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What is the problem?

In certain disorders the binding of endocannabinoids to CB1 receptors can worsen the disease obesity, for example; however in other disorders – such as multiple sclerosis (MS) – the opposite is true because the endocannabinoids have a protective effect. It may be beneficial if the activation of CB1 receptors by endocannabinoids could be enhanced or inhibited by drugs. Discovery of an allosteric site (a site different from the usual binding site) on the CB1 receptor has made this a possibility.

What are we interested in?

With a view to identifying the 'enhancers' (Positive Allosteric Modulators or PAMs) as well as the 'inhibitors' (Negative Allosteric Modulators or NAMs) of CB1 activation, a variety of drugs were tested. Identification of PAMs and NAMs (with regards to the CB1 receptor) could potentially lead to the discovery of drugs used in the body to treat disorders of the brain such as MS.

What did we do?

Tissue from mice was used to identify potential PAMs/NAMs at the CB1 receptor; vas deferens tissue was used due to the high concentration of CB1 receptors it contains. Two compounds, GAT211 and ORG, were tested to determine whether they displayed signs of allosteric modulation of CB1 receptor activation by anandamide, CP55940, WIN or THC. This was done by measuring any differences in the size of electrically-evoked contractions of the mouse tissue when comparing the effect of a CB1 receptor agonist alone, with its effect in the presence of a PAM/NAM added before the CB1 receptor agonist. We were also interested in seeing whether either of the two potential allosteric modulators behaved differently at the CB1 receptor depending on the agonist used, a phenomenon called 'probe-dependence'.

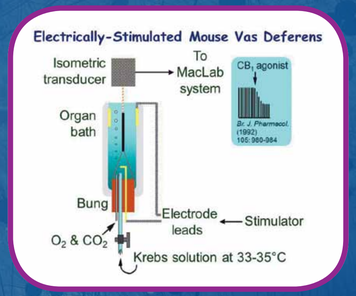

Diagram illustrating the experimental set-up

How did we do it?

The vas deferens tissue was placed in an organ bath and then subjected to electrical stimulation using 0.5-sec trains of 3 pulses. To begin with, the tissues were alternately stimulated (2 minutes) and rested (5 minutes) until the contractions stabilised. The potential PAM/NAM or control solution was applied and 13 minutes later, the tissue was subjected to a 2-minute period of electrical stimulation. A CB1 receptor agonist was administered at a concentration which was believed to produce its greatest inhibitory effect on electrically-induced contractions. After 13 minutes, the tissue underwent a further 2-minute period of electrical stimulation. The percentage inhibition of electrically-evoked contractions was also measured at 30, 45, 60 and 75 minutes after administration of the CB1 receptor agonist.

What did we find?

From Figures 1A to 1D it can be concluded that GAT211 acted as an anandamide and CP55940 PAM. However, it did not alter the effect of WIN or THC, perhaps due to probe-dependence.

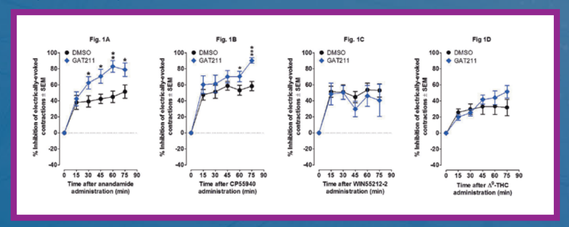

Figure 1A - 1D

ORG acted as a NAM of anandamide, CP55940 and WIN, but not of THC (see Figures 2A to 2D).

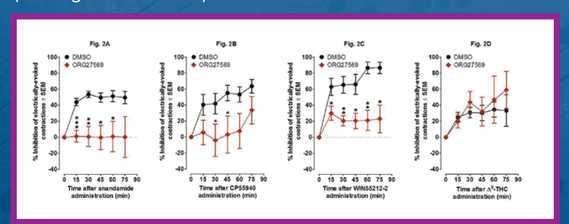

Figure 2A - 2D

The doses of anandamide CP55940, WIN and THC that were used in these experiments were those that we had found in our initial experiments to produce the greatest inhibitory effects of these compounds on electrically-evoked contractions.

What's next?

It will be important to investigate whether it is also possible to produce signs of allosteric modulation of the CB1 receptor in the whole body. Thus, for example, in diseases such as MS, we would expect that increasing endocannabinoid-induced activation of CB1 receptors would decrease debilitating side effects such as muscle spasticity. Therefore, discovery of a CB1 PAM which worked in the whole body could be beneficial.

Who am I?

I am now in the final year of a degree in Pharmacology at the University of Aberdeen and am grateful to Medical Research Scotland for letting me be part of a real research project. Not only will the research project help me with my honours year, it has also led me to decide which career I wish to pursue. Scientific research is not only interesting but crucial and although it is not a career I wish to follow, without this experience I would still be undecided about which career path to take.

Chelsea was supervised during her Vacation Research Scholarship project, *"A search for novel positive and negative allosteric modulators of the cannabinoid CB1 receptor"*, by Professor Roger Pertwee.