Cannabinoid signaling mechanisms

in the central nervous system

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Declaration of Originality

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ABSTRACT

A group of substances, known as cannabinoids, exist in the cannabis plant and have recently shown great promise as therapeutics for a wide range of conditions. A structurally related group of compounds known as endocannabinoids have also been found in the body. These compounds act at the same receptor as cannabis and exert their effects within defined areas of the CNS. Most notably amongst these areas is the spinal cord dorsal horn, which plays a key role in the processing of nociceptive (painful) signals.

In this thesis I examined several aspects of cannabinoid signaling that may play a role in spinal cord pain processing mechanisms. The first series of experiments asked whether the cannabinoid and serotonergic systems interact via the phenomenon of G protein-coupled receptor (GPCR) dimerization (or oligomerization). This mechanism may be important in controlling the ultimate action of ligands that bind to GPCRs. The type 1 cannabinoid receptor (CB_1) and the type 2A serotonin receptor ($5HT_{2A}$) subtypes were chosen because both are GPCRs and play roles in nociceptive processing. Each receptor was isolated from total human brain RNA, cloned, amplified in *E.* coli, and expressed in Baby Hamster Kidney (BHK) cells. Fluorescent resonance energy transfer (FRET) analysis, which measures the proximity of two fluorescent molecules according to the transfer of resonance energy from a fluorescent donor protein to an acceptor fluorophore, was used to determine whether the CB_1 and $5HT_{2A}$ receptors formed complexes with themselves and/or each other.

This series of experiments showed that the CB_1 and $5HT_{2A}$ receptors could form homo- and heterodimers, and were thus capable of interacting and indeed influencing one another's signaling pathways. The link between dimerization and cellular physiology is currently not clear, however the phenomenon is reasonably prevalent and has both basic science and applied implications. At the basic sciences level proteins that dimerize often have related functions or are involved in the same physiological processes (Woolf & Linderman, 2004). Thus, dimerization needs to be considered in future quests for discovery of novel classes of dimerization-regulating drugs.

The ultimate aim of a second set of experiments, was to examine the action of a synthetic cannabinoid (methanandamide) on the two ligand-gated ion channels (glycine and $GABA_A$ receptors) that are important for inhibitory signaling in the dorsal horn of the spinal cord. These experiments were driven by three considerations: both receptor types have been implicated in the onset and maintenance of various pain states; reports of a specific or unique type of glycine receptor (GlyR) in the superficial laminae of the spinal cord; and recent evidence for a direct (allosteric) action of cannabinoids on GlyRs in oocytes and dissociated neurons.

I first carried out an extensive characterization of fast inhibitory neurotransmission in the superficial (SDH; laminae I-II) and deep laminae (DDH: laminae IV-VI) of the mouse spinal cord dorsal horn. These two spinal cord regions have well established, although largely separate, roles in processing sensory inputs arising in skin, muscle, joints and viscera. Whole cell patch clamp electrophysiology was used to compare the properties of GlyRs and $GABA_A$Rs on SDH and DDH neurons in transverse spinal cord slices.

Several important properties of the two receptors differed in the SDH and DDH. Specifically, glycinergic mIPSC amplitude was smaller, decay time was slower, and frequency was lower in SDH versus DDH neurons. In contrast, $GABA_A$ergic mIPSCs had similar amplitudes and frequencies, but their decay times were faster in DDH neurons. These data suggest GlyR-mediated inhibition is more important in deep regions of the dorsal horn, which preferentially receives peripheral inputs from axons with high conduction velocities. The existence of large and fast inhibitory inputs in the DDH would be well suited to modulate the effect of such inputs. In contrast, smaller and slower $GABA_A$R-mediated inhibition appears to be equally important in both superficial and deep regions of the spinal cord dorsal horn. These features suggest $GABA_A$R-mediated inhibition is more important for fine-tuning the effects of a functionally wider range of peripheral inputs.

I next tested whether physiologically relevant concentrations of the endogenous cannabinoid, methanandamide (methAEA - 5 μM), had any direct effects on synaptically located GlyRs and $GABA_A$Rs. For GlyRs, methAEA reduced mIPSC frequency in the SDH and DDH but had no significant effect on mIPSC amplitude, rise time, or decay time constant. These observations are consistent with the well-documented presynaptic action of cannabanoids via CB_1 receptor-mediated mechanisms. Because methAEA had no effect on mIPSC amplitude or kinetics I conclude there is no evidence for a ***direct*** effect of methAEA on synaptically located glycine receptors in either SDH or DDH neurons. For $GABA_A$Rs, methAEA significantly reduced mIPSC frequency and slowed rise time in the SDH and DDH but had no significant effect on mIPSC amplitude, or decay time. This suggests that

methAEA may have a direct effect or modulatory action on $GABA_A$Rs, specifically their kinetics, in SDH and DDH neurons.

Because glycinergic and $GABA_A$ergic mIPSC properties differed in the SDH and DDH, I also compared the subunit expression of GlyR and $GABA_A$Rs, and the CB_1 receptor using real-time RT-PCR (qPCR) in each spinal cord region. In the SDH, the α1 subunit of the GlyR was the most highly expressed, followed by β, α2 and α3. In the DDH α1 and β were highly expressed followed by α2 and α3. The expression of both α1 and α2 GlyR genes was higher in the DDH. Comparison of $GABA_A$R subunit expression showed levels of α1 and β2 genes differed in the two regions, again with higher expression detected in the DDH. In contrast to previous reports, using immunohistochemistry, I found CB_1 receptor expression to be significantly higher in the DDH. When combined with the mIPSC data, my qPCR data are consistent with the dominance of fast GlyR-mediated inhibition in the DDH. Finally, my qPCR data provide no support for a higher concentration of CB_1Rs existing in the pain processing (lamina I-II) regions of the spinal cord.