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# Signal transduction of drugs of abuse

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Drug abuse is a significant socioeconomic problem. In addition to direct human affliction, the use of drugs (such as opiates and cannabinoids) is a major factor in urban criminality and in the spread of infectious diseases. Moreover, both opiates and cannabinoids have important beneficial medical properties, but their use is currently restricted due to their addictive properties.

**Regulation of adenylyl cyclase by acute and chronic opiate exposure**

Acute stimulation of opiate receptors activates $G_{i/o}$ proteins, resulting in inhibition of adenylyl cyclase (AC), while chronic activation has been shown to increase AC activity. This phenomenon, referred to as AC superactivation, has also been demonstrated for other $G_{i/o}$-coupled receptors, and was proposed to play a role in opiate addiction.

Nine AC isozymes, which differ in tissue distribution and stimulation/inhibition patterns, are currently known. Utilizing transfected COS-7 cells, we demonstrated that inhibition and superactivation are isozyme specific: AC-I, V, VI and VIII are inhibited by acute and superactivated by chronic opiate exposure, while AC-II, IV and VII are stimulated by acute exposure and do not show superactivation; AC-III is not significantly affected. AC superactivation is of a general nature, and is observed with other $G_{i/o}$-coupled receptors (e.g. $CB_1$-cannabinoid, $D_2$-dopaminergic, and $m_2$- and $m_4$-muscarinic). $G_{\beta\gamma}$ dimers were found to have a role in superactivation, as $G_{\beta\gamma}$ scavengers prevented AC superactivation.

Two approaches were taken to investigate the role of AC molecular domains in superactivation. The first was to characterize splice variants of AC-VIII, and the second was to produce point mutations in AC-V and AC-I. We found that acute μ-opioid receptor activation inhibits AC-VIII-A and B but not C (which lacks most of the $C_{1b}$ domain). Agonist withdrawal after chronic treatment induced superactivation of all three splice variants, demonstrating that the $C_{1b}$ area is not critical for AC superactivation. Using the second approach, we found that several mutations in the $C_{1a}$ region (AC-V DNV469-471AAA, AC-V F481Y, and the corresponding AC-I F314Y mutation) led to reduced inhibition upon acute agonist treatment and reduced

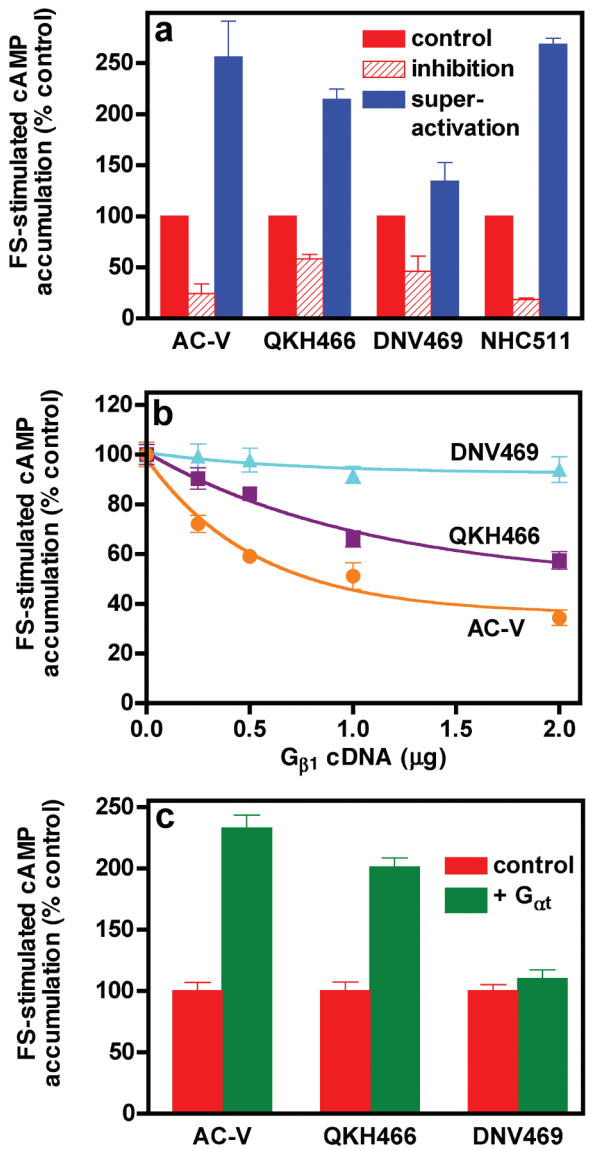


***Fig. 1*** *DNV469-471AAA mutation in AC-V reduces superactivation (a) and modulation by $G_{\beta\gamma}$ (b, c). COS-7 cells were transfected with μ receptor, AC-V or indicated mutants, and where indicated, $G_{\beta 1}$ or $G_{\alpha t}$ (a $G_{\beta\gamma}$ scavenger).*

superactivation. Moreover, contrary to wild type AC-V and AC-I, neither $G_{\beta\gamma}$ dimers nor constitutively active $G_{\alpha i}$ inhibited the activity of the mutated molecules, demonstrating that these amino acids in $C_{1a}$ play an important role in superactivation and AC interaction with both $G_{\beta\gamma}$ and $G_{\alpha i}$ protein subunits.

Future Plans: We plan to map the $G_{\beta\gamma}$ binding site(s) and study the role of $G_{\beta\gamma}$ in AC superactivation. Using the two-hybrid system, we will identify additional molecules that interact with AC and affect its activity following acute and chronic agonist exposure. We will also study the effects of chronic opiate exposure on other signaling pathways.

**Signaling by cannabinoid ligands**

In collaboration with Professor Raphael Mechoulam (Hebrew University, Jerusalem), we searched for endogenous materials in brain and other tissues which interact with cannabinoid receptors. This led to the discovery of a new cannabinoid ligand (2-arachidonyl glyceryl ether, or noladine), in addition to the two families of previously discovered endogenous cannabinoid ligands (anandamide and 2-arachidonoyl-glycerol). We found that all three endogenous cannabinoids bind and activate the "brain cannabinoid receptor" ($CB_1$), located on neural cells, and to a lesser extent the "peripheral cannabinoid receptor" ($CB_2$), expressed in cells of the immune system. Various mutations were inserted into the $CB_2$ receptor to study the role of various structural parts of the receptor in cannabinoid binding and signal transduction.

Future Plans: Characterizing derivatives of endogenous and tricyclic cannabinoids should expand our understanding of the functions of the $CB_1$ and $CB_2$ receptors, as well as of the vanilloid receptor (VR1 = capsaicin receptor), to which anandamide was recently shown to bind and activate. This could lead to the development of receptor-selective novel cannabinoid drugs with beneficial properties (such as anti-inflammatory and antinociceptive agents, and drugs for treatment of glaucoma).

***Selected Publications***


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***For additional information see:***

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