Drug abuse is one of the most notorious socioeconomic problems of our time. In addition to the direct human affliction, the use of drugs of abuse (such as opiates and cannabinoids) is a major factor in urban criminality and in the spread of infectious diseases, including AIDS. Moreover, both opiates and cannabinoids have important beneficial medical properties (e.g., morphine in surgery), but the use of such drugs is currently legally restricted due to their addictive properties, thus limiting their medical use.

A. Regulation of adenylyl cyclase by acute and chronic opiate exposure

Little is known about the molecular mechanisms by which chronic opiate exposure leads to the development of opiate tolerance and dependence, and to the subsequent phase of opiate withdrawal (which is the major reason for the reluctance by abusers to give up drugs).

Our goals are to characterize the biochemical changes accompanying chronic opiate treatment and the withdrawal phase. Along these lines, we were able to show (using CHO cells transfected with \( \mu \)- or \( \kappa \)-opioid receptor) that while acute exposure to opiate agonists leads to adenylyl cyclase (AC) inhibition, chronic exposure results in AC superactivation (particularly evident upon withdrawal of the inhibitory opiate agonist). Nine types of AC isozymes, which differ in their tissue distribution and in their stimulation/inhibition patterns, are currently known. Utilizing COS-7 cells transfected with \( \mu \)-opioid receptor and the individual AC isozymes, we found that the AC isozymes could be divided into three groups: (i) AC-I, V, VI and VIII are inhibited by acute and superactivated by chronic opiate exposure, with AC-V (known to be localized to brain areas involved in drug addiction) yielding the largest superactivation; (ii) AC-II, IV and VII are stimulated by acute opiate exposure and do not show superactivation; and (iii) AC-III is not significantly affected by opiate exposure. Moreover, we showed that the phenomenon of AC superactivation is of a general nature, and AC-I, V, VI and VIII are superactivated following chronic activation by agonists of other \( G_{i/o} \)-coupled receptors (e.g., CB1 cannabinoid, \( D_1 \)-dopaminergic, and \( m_2 \) - and \( m_3 \)-muscarinic).

The second step of this research work involved investigating the role of \( G_{i/o} \) dimers (released from \( G_{i/o} \) upon receptor activation) in AC regulation. Using transfected COS cells, we confirmed that AC-I is inhibited and AC-II is stimulated by \( G_{i/o} \). However, we found that different \( G_{i/o} \) subunits differ in their regulation of AC activity, with \( G_{i/o} \) stimulating, and \( G_{i/o} \) (an isoform abundant in brain) inhibiting AC-II. \( G_{i/o} \) was also found to inhibit AC-V and VI (a weaker inhibition was observed with \( G_{i/o} \)). Moreover, \( G_{i/o} \) scavengers increased the activity of AC-V and VI and prevented AC superactivation, suggesting that endogenous \( G_{i/o} \) tonically inhibits the activity of these AC isozymes, and that this inhibition is reversed by chronic agonist exposure (Fig. 1). To determine whether the effect of \( G_{i/o} \) on AC-V is direct or indirect, we showed that anti-\( G_{i/o} \) antibody co-immunoprecipitated AC-V, suggesting a direct interaction. Moreover, we found that \( G_{i/o} \) interacts with the \( G_{i/o} \) intracellular loop of AC-V, and have localized the binding area to a fragment of 65 amino acids. A specific mutation in this area abolished \( G_{i/o} \) binding. Moreover, when this mutation was introduced into AC-V, its capacity to be superactivated was markedly reduced, suggesting that \( G_{i/o} \) binding to this area is important for superactivation.

Future Plans: Employing molecular biological techniques, we plan to map the \( G_{i/o} \) binding site(s) and to study the role of the \( G_{i/o} \) subunits in AC superactivation. We will also study the effects of chronic opiates on other signaling pathways.

B. Properties of endogenous 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 search led to the discovery of two families of endogenous cannabinoid ligands, including that of anandamide (arachidonoyl-ethanolamide) and of 2-arachidonoylglycerol. We found that these materials bind and activate the “brain cannabinoid receptor” (CB1), located on neural cells, and to a lesser extent the “peripheral cannabinoid receptor” (CB2), expressed in cells of the immune system.
More recently, we assayed derivatives of the endogenous ligands, as well as of the tricyclic cannabinoids, for their biological and pharmacological properties. We also found that the endogenous cannabinoids are present in body tissues together with additional pharmacologically inactive (or partially active) acylamides and 2-acyl-glycerol esters. We have established that these “entourage compounds” enhance receptor binding of the endogenous ligands, stabilize them against hydrolysis, and potentiate their in vivo and in vitro effects. We are currently screening these materials for their biological roles and therapeutic effects.

Future Plans: Characterizing derivatives of endogenous and tricyclic cannabinoids should expand our understanding of the functions of the brain and peripheral cannabinoid receptors, and lead to the development of receptor-selective novel cannabinoid drugs with beneficial properties (such as anti-inflammatory agents and drugs for treatment of glaucoma).

Selected References


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Fig. 1. Gβγ in AC-V regulation—Acute agonist activation leads to AC inhibition mediated by Gαi and Gβγ, while chronic treatment leads to loss of Gβγ inhibition and to AC superactivation. Agonist withdrawal leads to “overshoot” of AC activity. Inhibitory signals are indicated by blunted lines. AC activity is indicated by the number of arrows presented between the C domains of AC.