Perspectives in Pharmacology

Identification of the Endogenous Cannabinoid System through Integrative Pharmacological Approaches

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ABSTRACT

Scientific progress in the biological sciences increasingly relies on an integration of behavioral, pharmacological, cellular, and molecular approaches, particularly in translating basic research observations into therapeutic potential. The strength of in vivo model systems lies in the direct assessment of physiological function. However, they only allow indirect evidence for mechanism of action. Frequently, in vitro models provide just the opposite. A combination of both in vitro and in vivo approaches are often essential for establishing the underlying mechanisms of a specific pharmacological effect. In recent times, an endogenous cannabinoid system has been characterized due to the combined efforts of chemists, pharmacologists, molecular and cellular biologists, and biochemists. This endogenous cannabinoid system is providing a basis for systematically addressing the pharmacological controversies surrounding marijuana. The description of this endogenous cannabinoid system and the strategies for establishing the physiological function of this system are the subjects of this article.

Integrative Pharmacology

Scientific knowledge is accruing at a rate unparalleled in history. In recent times, we have learned a great deal about basic physiological and biochemical processes and the ensuing pathology when these processes are disrupted either temporarily or permanently. We typically define biological systems on the basis of a specific substance and often in such a manner to suggest that these systems are discrete entities. The challenge of understanding a specific system, such as any one of the neurotransmitter systems, is magnified when that system is considered in context with all of the other possible biological systems with which it may communicate. Although the ordering of multiple cell types provides the basic biological scaffolding, it is the cell-cell signaling that provides functionality. Moreover, each organ has a unique composition of cells and a specific manner in which they communicate. Further, all of the organs must act in concert to maintain the whole animal. With our increased knowledge has come a greater appreciation for biological complexity and the challenges ahead. For many diseases, better treatments are closer at hand, and yet, cures remain as elusive as ever.

There have always been interdisciplinary approaches to solving biological complexities. In fact, pharmacology was born when physiologists began studying discrete chemicals on intact biological systems. Early pharmacologists relied on the chemist to provide the chemical tools, the biochemist to identify specific biological pathways, the clinician to identify the malady, and so on. Interactions among scientific disciplines will always be essential despite the fact that scientific progress has driven specialization within each of the disciplines, including pharmacology. Although it is possible to make enormous strides using a limited set of approaches, it has become abundantly clear that there is a greater likelihood of success with the integration of multiple approaches. There is no question that studying a mutated receptor in an expression system can reveal much about the nature of ligand/receptor interactions, but the question of physiological relevance remains. Conversely, administration of a drug to a whole organism may reveal much about physiological and pharmacological relevance but only indirect evidence for mechanism of action. Combining these two approaches can be much more powerful. Therein lies the reason we are wit-

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ABBREVIATIONS: THC, Δ^9-tetrahydrocannabinol; CB₁, cannabinoid receptor; SR 141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride; WIN 55,212-2, (R)-(-)[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-d,e]-1,4-benzoxazin-6-yl]-1-naphth-2-yl)methanonesulfonate.
nessing a greater integration of pharmacological approaches to address scientific questions. Marijuana research represents an excellent example of how multiple scientific disciplines converged to provide a biological explanation for the complex pharmacological properties produced by this plant material.

Early Marijuana Studies: A History of Challenges

During the past decade, enormous progress has been made toward understanding the mechanisms through which marijuana produces many of its effects. At the same time, a considerable number of questions remain unanswered. This progress has come about because of an integration of pharmacological approaches that has successfully related cellular biological events to in vivo pharmacological effects. A logical question is why a substance such as marijuana could be studied so extensively in the past, and yet, its mode of action could remain an enigma for so long. There are several contributing factors. As with most plant materials of pharmacological interest, the initial characterization was confined to self-reports by individuals who experimented with cannabis. Moreover, marijuana is typical of most psychoactive substances in that it produces a myriad of behavioral and pharmacological effects, many of which are qualitatively and quantitatively dissimilar among users. Some of the most prevalent effects include euphoria or a sense of well being, sedation, dream-like state, distortion of sensory perceptions and elapsed time, disruption of cognitive functioning, and impairment of fine motor skills (Martin, 1995). The most consistent pharmacological effect is tachycardia, which may or may not be accompanied by orthostatic hypotension. The description of the pharmacological effects in humans can be attributed to extensive self-reporting by recreational users of marijuana and by carefully controlled studies by psychopharmacologists and other clinicians. These descriptions were vital in that they strongly suggested that marijuana was a highly potent and unique psychoactive substance.

Establishing the pharmacological nature of marijuana’s effects is not easily accomplished in humans because of the subjective nature of many of its effects and the fact that only limited mechanistic studies can be conducted in humans. The use of lab models offers a logical substitute for human experimentation, but subjective measures cannot easily be mimicked in laboratory animals. Moreover, plant materials always pose problems for experimental biologists. Uncertainties over drug stability, dosimetry, combination of active and inactive constituents, etc. lead to conflicting reports and cloud interpretation of data. For most psychoactive plants, such as the opium poppy, coca, and tobacco, the active constituents were isolated, identified, and synthesized for experimental studies long ago. With marijuana, its primary psychoactive constituent, THC, was not definitively identified until 1964 (Gaoni and Mechoulam, 1964). It turned out that marijuana was not a very good source of THC because of the difficulty of isolating and purifying it from marijuana. THC was not available to the general scientific community until a reasonable synthetic pathway was developed that allowed the preparation of sufficient quantities (Razdan et al., 1972). Therefore, chemists played a pivotal role in the identification and preparation of a reliable source of material for scientific study. Despite all of these early hurdles, substantial progress has been made in characterizing the pharmacological effects of marijuana and synthetic cannabinoids in humans and laboratory animals, and an endogenous cannabinoid system has been identified (Fig. 1). These accomplishments are due to the combination of chemistry, behavioral pharmacology, cellular pharmacology, neuroscience, molecular biology, biochemistry, and structural biology. The result has been the identification of an endogenous cannabinoid system composed of two receptor subtypes, signal transduction pathways and endogenous ligands along with synthetic and degradative pathways for the endocannabinoids. Moreover, evidence is now emerging about the potential physiological relevance of this endogenous system.

Cannabinoid Receptors

Although the availability of THC represented a milestone in cannabinoid research, difficulties remained. THC is a highly lipophilic resinous material, a property that complicated both in vivo and in vitro studies. As a result of its
pharmacokinetics properties, THC proved to be highly potent in vivo but was weakly active in most in vitro situations. Its high lipophilicity, broad spectrum of action, and low in vitro potency led to early speculation that cannabinoids merely intercalated into cell membranes to disrupt normal physiological processes. Its high in vivo potency and unique pharmacological profile, however, were much more consistent with a receptor mechanism than an anesthetic-like membrane perturbation. Early synthetic efforts by several different chemists led to a large number of structurally related THC analogs (Razdan, 1986). Of course, there had to be models to test these compounds for cannabinoid activity. Models that proved useful for assessing pharmacological potency of cannabinoids include the dog-static ataxia test, rat and monkey drug discrimination, and the tetrad test in mice (depression of spontaneous activity, antinociception, hypothermia, and catalepsy) (Razdan, 1986). Working together, chemists and behavioral pharmacologists were able to establish a strict structure-activity relationship for cannabinoids that established the basis for a receptor mechanism (Pertwee, 1999). For example, the synthesis of the enantiomers of 11-OH-THC-dimethylheptyl revealed that the (-)-isomer was extremely potent and several hundred times more potent than the corresponding (+)-isomer (Mechoulam et al., 1988). Moreover, lipophilicity was found not to be associated with pharmacological potency (Thomas et al., 1990). The characterization of a high-affinity cannabinoid binding site (Devane et al., 1988) and its distribution in brain (Herkenham et al., 1990) preceded the actual cloning of a G protein coupled receptor (Matsuda et al., 1990) that turned out to be a cannabinoid receptor (CB1). Several lines of evidence suggest that this single receptor is responsible for the central effects of cannabinoids. First, there is an excellent correlation between CB1 cannabinoid receptor affinity and in vivo potency for cannabinoid analogs (Compton et al., 1993). Second, SR 141716A, is an effective cannabinoid antagonist and highly selective for the CB1 cannabinoid receptor (Rinaldi-Carmona et al., 1994). Third, animals in which the CB1 receptor has been deleted do not produce most cannabinoid effects when administered THC (Ledent et al., 1999; Zimmer et al., 1999). Fourth, there is only one additional cannabinoid receptor subtype (CB2) known at present, and it is confined to the periphery (Munro et al., 1993). Establishing the biological relevance of CB1 receptors required the integration of chemistry, general pharmacology, neuroscience, molecular biology, and cellular biology. Since the physiological and pharmacological significance of CB2 receptor has yet to be fully determined, this article will concentrate on CB1 receptors.

**Signal Transduction**

Howlett’s laboratory, using structure-activity relationship studies, provided the first compelling evidence that a putative cannabinoid receptor was linked to G proteins (Howlett and Fleming, 1984), an observation that was confirmed when the receptor was cloned (Matsuda et al., 1990). There is strong evidence for CB1 receptor coupling to G\textsubscript{i/o} proteins, and recent studies revealed CB1 receptor coupling to multiple G\textsubscript{i} proteins (Prather et al., 2000). Although there is also some evidence for CB1 receptor coupling to G\textsubscript{q} proteins (Glass and Felder, 1997), the predominate effects of cannabinoids occur through inhibitory G protein function, including inhibition of adenylyl cyclase, inhibition of calcium channels (N- and Q-types), and activation of inwardly rectifying potassium channels (Mackie and Hille, 1992; Mackie et al., 1995). In addition, mitogen-activated protein kinases are activated by the CB1 receptor (Bouaboula et al., 1995). It is not yet clear whether all signal transduction systems are activated simultaneously and the extent to which they are involved in specific cannabinoid actions. However, cellular pharmacology has provided ample evidence that these transduction pathways are activated by CB1 cannabinoid receptors.

**Endogenous Ligands**

The identification of endogenous substances in brain capable of binding to CB1 receptors provided the first evidence that cannabinoid receptors are not vestigial. Mechoulam’s laboratory has now identified three classes of arachidonoyl derivatives that include the amide anandamide (Devane et al., 1992), the ester 2-arachidonoyl-glycerol (Mechoulam et al., 1995), and the 2-arachidonoyl glyceryl ether (Hanus et al., 2001). These endogenous substances are considered endocannabinoids because they activate CB1 cannabinoid receptors, produce effects that are consistent with CB1 cannabinoid receptor activation, and synthetic and degradative pathways have been identified. There is substantial evidence that a calcium-dependent, energy-independent transacylase transfers arachidonic acid from the sn-1 position of phosphatidylethanolamine to the amino group in phosphatidylethanolamine, with subsequent hydrolysis by a phospholipase D-type enzyme to form anandamide (Schmid, 2000). Inactivation of anandamide occurs primarily by fatty acid amide hydrolase, an enzyme that has been cloned (Patricelli et al., 1998). Blockade or deletion of this enzyme in mice greatly potentiates the actions of exogenously administered anandamide (Cravatt et al., 2001). Anandamide may well be inactivated in part through a specific uptake mechanism. Anandamide is transported across cellular membranes by a protein-mediated process that has the characteristics of facilitated diffusion, is bi-directional, and sodium- and ATP-independent (Hillard and Jarrahian, 2000). Although the pharmacological characteristics of this transport mechanism have been reasonably well characterized, its molecular structure and biological functions remain a mystery. Our knowledge of the synthesis and degradative pathways of endocannabinoids has come from the biochemists and molecular biologists. The major challenge is to elucidate the physiological stimuli that regulate the endocannabinoid enzymes and transporters. Elucidation of the regulation of the endocannabinoid system is key to understanding its role in both normal and pathophysiological states.

**Role of the Endogenous Cannabinoid System**

**Pain Perception.** Reviewing the historical literature, there are abundant references to the use of marijuana for controlling the symptoms arising from almost any malady. Although much of this literature must be interpreted cautiously because of its anecdotal nature, a reasonable interpretation of such disparate therapeutic indications is general symptom management rather than disease-specific treat-
ment. Common symptoms of these maladies include discomfort and pain, and indeed, marijuana, marijuana extracts, and synthetic cannabinoids have been reported to be analgesic agents (Martin and Lichtman, 1998; Richardson, 2000). In brief, marijuana efficacy for controlling pain is equivocal with some studies reporting analgesic effects, whereas others fail to do so. On the other hand, there is little doubt that THC and synthetic analogs are effective analgesic agents in humans and laboratory animal models. Unfortunately, cannabinoid analgesia or antinociception occurs at doses that also usually produce other central nervous system effects. This coincidence of analgesic and nonanalggesic effects fueled speculation that cannabinoids were not truly analgesic agents but rather were compounds that merely confounded the perception of pain in humans or the detection of nociception in animals through nonspecific actions.

The identification of the endogenous system provided an opportunity to determine whether cannabinoids play a unique role in pain perception. First, cannabinoid agonists are effective in a wide range of acute and chronic pain models that include thermal- and chemical-induced nociception (Walker et al., 1999). The CB1 receptor was implicated that include thermal- and chemical-induced nociception are effective in a wide range of acute and chronic pain models. Unfortunately, cannabinoid analgesia or antinociception occurs at doses that also usually produce other central nervous system effects. This coincidence of analgesic and nonanalggesic effects fueled speculation that cannabinoids were not truly analgesic agents but rather were compounds that merely confounded the perception of pain in humans or the detection of nociception in animals through nonspecific actions.

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tenuate low-magnesium-induced epileptiform discharges in rat hippocampal slice preparation (Ameri et al., 1999). These authors concluded that CB₁ cannabinoid receptors and anandamide control neuronal excitability by reducing excitatory neurotransmission at a presynaptic site, a mechanism that might prevent excessive excitability that would otherwise lead to epileptiform activity. The mechanism underlying this dampening of excitability is believed to involve inhibition of presynaptic excitatory neurotransmitter release of which glutamate is the most ubiquitous. Further support for a CB₁ cannabinoid receptor role was provided by studies showing that SR 141716A inhibited the cannabinoid-induced attenuation of neuronal excitability (Rinaldi-Carmona et al., 1994).

It has been suggested that the mechanisms by which THC and WIN 55,212-2 decrease hyperexcitability in in vitro models involve CB₁ receptor modulated ion channels, as discussed briefly above. Extensive molecular and pharmacological studies have shown that agonist binding to the CB₁ receptor activates an inhibitory G protein leading to decreased production of cAMP and thus reduced activity of the enzyme protein kinase A, known to modulate the activity of several ion channels. The CB₁ receptor-mediated increase in rectifier and A-type potassium currents serve to stabilize neuronal membrane potential and make the cell less likely to manifest seizure activity. In addition, CB₁ receptor activation produces a decrease in N- and P/Q-type voltage-gated calcium currents. The subsequent reduction in presynaptic intracellular calcium load causes a decrease in calcium-dependent neurotransmitter, most notably glutamate. This amino acid is the primary excitatory neurotransmitter in the central nervous system and elevated levels have been found in human epileptogenic foci. An attenuation of glutamate release would theoretically prevent seizure spread by synaptic transmission from an epileptic focus to the rest of the brain.

Emesis. It is reasonable to assume that nausea and vomiting represent basic mechanisms through which some mammals are able to void substances that make them ill. Nausea and vomiting are common to many disease states, and there are numerous emetogens, such as cytotoxic drugs (chemotherapeutic agents), radiation, and opioids. Although nausea and vomiting suppress appetite, the processes do not depend upon a common neural circuitry. In contrast to the predominant role of the hypothalamus in appetite, the area postrema-nucleus tractus solitarius in the brainstem plays an essential role in emesis. Additionally, the dopaminergic, cholinergic, and serotonergic systems in the gastrointestinal tract can play a role in emesis. The discovery by young patients that smoking marijuana before undergoing chemotherapy relieved the ensuing nausea and vomiting led to clinical trials demonstrating the efficacy of THC. Several cannabinoids have proven to be effective in blocking cisplatin- and apomorphine-induced emesis in a variety of animal species, most recently in the least shrew (Darmani, 2001a).

Although THC was approved for use in controlling chemotherapy-induced emesis in the mid 1980s, its mechanism of action was unknown. Evidence is now emerging that the endogenous cannabinoid system is directly involved. Typically, models of emesis involved nonrodent models, such as dogs, that made mechanistic studies difficult because they were labor-intensive and controversial. The least shrew (Cryptotis parva) represents an ideal alternative experimental model of vomiting (Darmani, 2001b). SR 141716A induces vomiting in the shrew, and various cannabinoid agonists blocked this effect. Activation of the cannabinoid system is also effective in blocking opioid-induced vomiting in ferrets (Simoneau et al., 2001). The CB₁ cannabinoid receptor is involved since SR 141716A will block the action of cannabinoid agonists in this model. Presently, there is ample clinical data to demonstrate THC antiemetic efficacy and supportive data in laboratory animal models. Therefore, it is expected that these cannabinoid effects are mediated through the CB₁ cannabinoid receptor. It remains to be established whether the endocannabinoids serve as primary mediators of emesis, exert a modulatory influence on those that do, or work through a combination of direct and indirect actions.

Appetite. One of the most notable effects of cannabis and synthetic cannabinoids is appetite stimulation. It was these observations that led to approval of THC for treating acquired immunodeficiency syndrome-related cachexia. Until recently, there was no evidence for a direct action of cannabinoids on anorexigenic/orexigenic pathways. Central and peripheral pathways are involved in the regulation of appetite and energy stores (Chiesi et al., 2001). Although a large number of neuropeptides, hormones, and monamines have been implicated as modulators of food intake, considerable attention has been directed toward leptin, which is known to reduce food intake. Briefly, leptin secreted by adipose tissue acts within the hypothalamus at the arcuate nucleus to suppress appetite-stimulating peptides (neuropeptide y and agouti-related protein) and stimulate the activity of appetite-reducing peptides (α-melanocyte-stimulating hormone and cocaine- and amphetamine-regulated transcript). In addition to the observation that cannabis and endocannabinoids stimulate food intake, it is important to note that the hypothalamus contains both CB₁ receptors and anandamide and 2-arachidonoyl glycerol. Recently, it was reported that acute treatment with leptin reduces the levels of anandamide and 2-arachidonoyl glycerol. Currently, it is reported that acute treatment with leptin reduces the levels of anandamide and 2-arachidonoyl glycerol in the hypothalamus of normal rats and that these endocannabinoids are elevated in obese leptin-deficient ob/ob and obese leptin-receptor deficient db/db mice (Di Marzo et al., 2001). It was also shown in this investigation that following food restriction, CB₁ receptor knockout mice ate less than wild-type mice did. Accordingly, SR 141716A reduced food intake in the wild-type but not CB₁ knockout mice. These studies suggest that the endocannabinoid system plays an active role in regulating feeding behavior. Furthermore, this role seems to directly involve the neural circuitry regulated by leptin rather than a general euphorogenic action. Although THC is already marketed for appetite stimulation and SR 141716A is in clinical trials for weight reduction, establishing the precise mechanism through which these agents modulate food intake opens new avenues for intervention strategies.

Vascular Function. An excellent perspective on the role of endocannabinoids in vascular function was presented recently (Hillard, 2000). The emphasis of this article was the mechanisms through which cannabinoids produce vasodilation and hypotension. Cannabinoids also produce tachycardia in humans, so that the hypotensive effects are not always observed unless high quantities of marijuana are smoked or ingested. The possible mechanisms for vascular dilation include inhibition of transmitter release from sympathetic nerve terminals, direct effects on vascular smooth muscle
cells, and effects on endothelial cell function. Hillard (2000) summarized the literature that suggests CB1 receptors located on axon terminals of sympathetic neurons decrease calcium influx or increase potassium channel opening with a resultant decreased neurotransmitter release. Cannabinoid agonists, including the endocannabinoids, produce hypotension that is blocked with SR 141716A and absent in CB1 receptor knockout mice.

Although most of the interest in cannabinoid-induced alterations in these vascular effects can be traced directly to a neuronal site of action, a compelling argument for non-neuronal sites of action can be made. Cannabinoids produce vasorelaxation of cat cerebral arteries and inhibit L-type calcium channels in cat cerebrovascular smooth muscle cells that express the CB2 cannabinoid receptor. The cannabinoid role in other vascular beds seems to be different and less well defined. Endothelial cells seem to be involved in these vasodilatory effects that may involve both a CB1 and non-CB2 receptor. Evidence for the latter includes anandamide-induced endothelial-dependent vasodilation that is SR 141716A-sensitive in the mesentery; however, this effect is not produced by other cannabinoid agonists. This effect of anandamide is retained in CB1 receptor knockout mice. Perivascular sensory nerve endings in the mesentery also release endocannabinoids. The significance of this emerging characterization is that endocannabinoids regulate peripheral processes. Moreover, the endocannabinoid system remains to be fully characterized, particularly in light of the possibility that additional receptor subtypes may exist (Jarrai et al., 1999; Breivogel et al., 2001).

Summary

The convergence of scientific contributions from multiple disciplines led to the identification of an endogenous cannabinoid system. A putative role for this system in selected physiological processes was summarized above. Without the integration of multiple scientific approaches, progress would have been modest and limited. The initial observations that marijuana produced unique behavioral and pharmacological effects in animals and laboratory animals prompted chemists to prepare synthetic agonists and antagonists. These tools provided the foundation for molecular biologists to clone receptors, neuropharmacologists and electrophysiologists to assess signaling pathways, chemists to isolate endogenous ligands, biochemists to identify synthetic pathways, and scientists with divergent interests to assess the functionality of the resulting biological system.

References


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