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COMMENTARY

Role of Cyclic AMP in the Actions of Cannabinoid Receptors

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ABSTRACT. Cannabinoids, including Δ^9-tetrahydrocannabinol (THC), bind to receptors that couple to $G_{i/o}$-proteins and inhibit adenylyl cyclase. However, like other G-protein-coupled receptors, cannabinoid receptors are also coupled to other effector systems. This review examines the characteristics of the cannabinoid–G-protein–adenylyl cyclase system, and explores the role of cyclic AMP in mediating effects of these drugs. Several conclusions emerge from this research. First, the principal actions of cannabinoids are mediated through G-protein-coupled receptors, and the intracellular signaling mechanism that initiates cellular response of cannabinoids is activation of G-proteins. Second, cannabinoid-inhibited adenylyl cyclase is only one of several different effectors coupled to these receptors, and different effectors may be used for different types of responses. Third, cannabinoid inhibition of adenylyl cyclase plays an important role in several aspects of cannabinoid function, including modulating conductance at a voltage-dependent K^+ channel ("A" current) in the hippocampus, thus providing an effective rationale for behavioral effects of cannabinoids mediated in this region. Other functions of this system may include production of long-term changes in gene expression by inhibition of cyclic AMP response elements on strategic genes, and inhibition of anandamide synthesis, thus mediating some of the long-term effects of cannabinoids on neuronal function. BIOCHEM PHARMACOL 52;6:819–827, 1996.

KEY WORDS. Δ^9-tetrahydrocannabinol; G-proteins; K^+ channels; cyclic AMP; protein kinase

cAMP† has played a pivotal role in developing our understanding of cannabinoid receptors, since the discovery that cannabinoids inhibited adenylyl cyclase [1] was the basis of the concept that cannabinoid actions are mediated through G-protein-coupled receptors. Ever since Gaoni and Mechoulam [2] found that the principal psychoactive constituent of the cannabis plant was THC, much effort has been spent in determining the mechanisms of action of this psychoactive drug. For years, two contrasting theories competed to explain the mechanism of action of THC [3, 4]. One theory held that THC binds to specific receptors, analogous to those already observed for other drugs of abuse such as cocaine and opioids. A wealth of pharmacological data indicated a specific structure–activity relationship for cannabinoids [4], and supported the concept of a receptor-mediated mechanism of action. However, the high degree of lipophilicity of these drugs made them technically difficult to utilize in radioligand binding assays and, despite much effort, no biochemical evidence for specific cannabinoid receptors was available. Because of this lack of concrete information, some groups favored a second hypothesis: that cannabinoids act as lipophilic drugs to alter membrane fluidity and thus affect neuronal membrane potentials [5]. Although some biophysical evidence supported this hypothesis, the pharmacology of this effect generally did not correspond to that of the behavioral structure–activity studies of these drugs. Therefore, by the mid-1980s, after over 20 years of research, there was no consensus on the mechanism of action of cannabinoids.

The breakthrough in our understanding of these drugs came from two independent developments. First, researchers at Pfizer synthesized THC analogs that were several hundred times more potent than THC in behavioral assays [6]. Since THC itself is relatively weak in binding to its receptor, the development of these potent cannabinoid analogs provided the pharmacological tools that could be used in distinguishing specific from non-specific binding. The first group of these potent analogs is represented by CP-55,940, commonly used as a radioligand in cannabinoid receptor binding assays (Fig. 1). Another structurally unrelated series of analogs are the aminoalkylindoles, represented by WIN 55212-2 (Fig. 1), which is not only useful as a radioligand [7] but also widely available as a potent agonist for *in vitro* studies [8]. The second development occurred when Howlett showed that cannabinoids decrease cAMP by inhibiting adenylyl cyclase in neuroblastoma cell cultures [1]. This effect was pharmacologically relevant and pertussis toxin sensitive, thus confirming that the response

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† *Abbreviations:* cAMP, cyclic AMP; THC, Δ^9-tetrahydrocannabinol; [^{35}S]GTPγS, [^{35}S]guanylyl-5′-O-(γ-thio)triphosphate; CB1 and CB2, brain and peripheral cannabinoid receptors, respectively; CRE, cAMP-response element; and GABA, γ-aminobutyric acid.

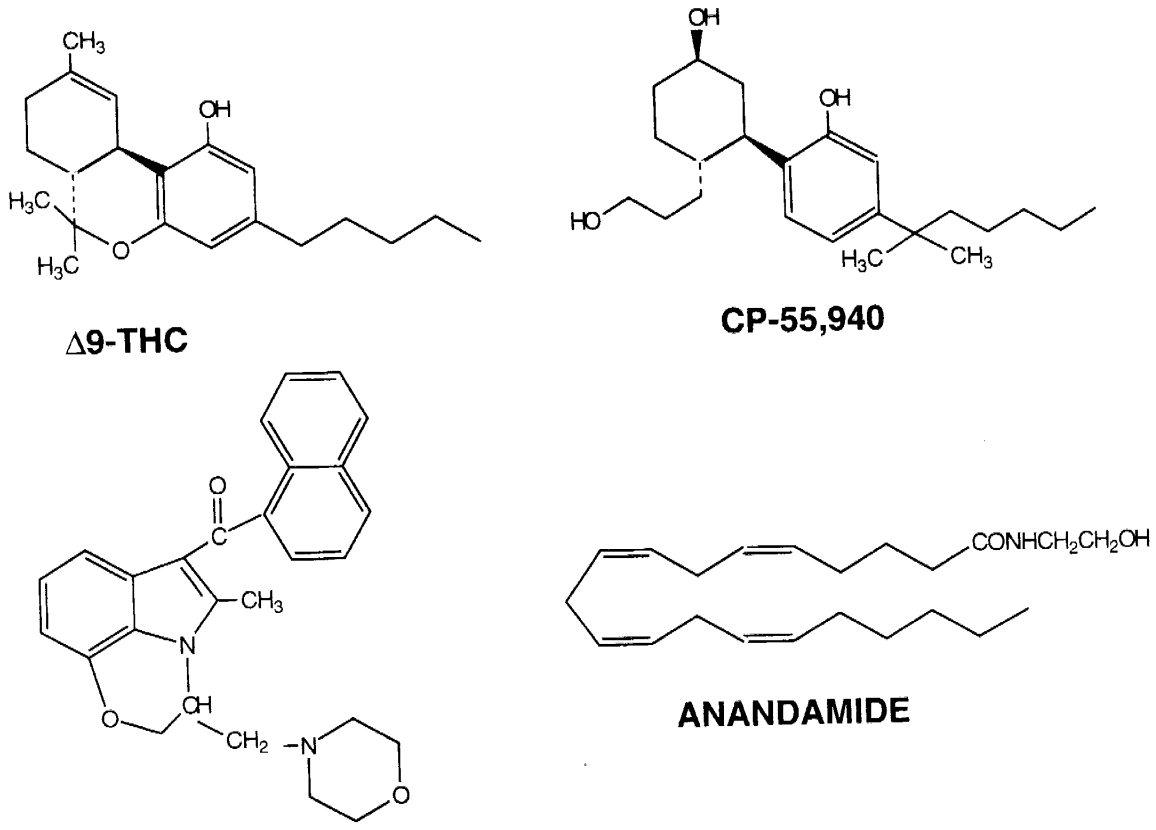

FIG. 1. Structures of common cannabinoids, comparing Δ^9-THC with two potent cannabinoid agonists, CP-55,940 and WIN 55212-2, along with the endogenous cannabinoid anandamide.

was mediated by a receptor from the $G_{i/o}$-coupled superfamily of receptors [9–11]. This finding established the existence of authentic cannabinoid receptors as members of a recognized family of receptors with clearly recognized biochemical properties, and it was followed rapidly by the development of a cannabinoid receptor radioligand binding assay [12], characterization of cannabinoid receptor localization in brain [13], cloning and sequencing of the seven-transmembrane structure of the brain cannabinoid receptor [14], and the subsequent identification of anandamide as an endogenous ligand for cannabinoid receptors [15]. More recently, the cloning of a peripheral cannabinoid receptor from spleen cells [16] confirms that there are at least two types of cannabinoid receptors, with the brain receptor type known as CB1, and the peripheral type known as CB2.

Nevertheless, despite the knowledge that cannabinoids bind to G-protein-coupled receptors, several questions about the role of these signal transduction mechanisms still persist. Are all of the actions of THC mediated through these G-protein-coupled receptors, or are there other (perhaps non-receptor mediated) mechanisms that help explain the psychoactive properties of THC? And, if these actions are mediated through G-protein-coupled receptors, what (if any) role does cAMP itself play in mediating these actions? Are there other effectors besides cAMP which may play even more important roles in mediating THC actions? This review will explore each of these questions by examining the biochemical, pharmacological, and electrophysiological properties of cannabinoid receptors in brain and specific cell culture systems.

G-PROTEIN-COUPLED ACTIONS OF CANNABINOIDS

Receptors coupled to $G_{i/o}$-proteins are involved with at least three effector systems: inhibition of adenylyl cyclase, increase in potassium conductance, and decrease in calcium conductance [17]. The finding that cannabinoid-inhibited adenylyl cyclase was sensitive to pertussis toxin [11], and the subsequent cloning of a seven-transmembrane receptor [14], confirmed that the signal of binding of a cannabinoid agonist to its receptor is mediated by activation of $G_{i/o}$-proteins. In other words, regardless of the effector involved, the initial step in the agonist response is activation of G-proteins. The mechanisms of receptor–G-protein activation have been reviewed elsewhere [17, 18], and cannot be reviewed in detail here. Briefly, each G-protein is a heterotrimer composed of α, β, and γ subunits. The Gα subunit binds and hydrolyzes GTP; binding of agonist to receptor increases affinity of Gα for GTP and decrease

affinity for GDP. GTP binding activates Gα and decreases its affinity for βγ, causing βγ to dissociate from Gα. The receptor and Gα then dissociate, enabling Gα to interact with effectors. The Gα subunit is inactivated by an intrinsic GTPase that hydrolyzes GTP to GDP, increasing the affinity of Gα for βγ. The reaction is catalytic, since each receptor can activate multiple G-proteins, which results in amplification of the receptor signal into an intracellular response [19, 20].

Receptor activation of G-proteins in isolated membranes can be measured by receptor-stimulated low K_m GTPase [21–23], or by receptor-stimulated [^{35}S]GTPγS binding. The latter technique is a direct assay of receptor activation of G-proteins, since it measures the activation reaction of the cycle, i.e. the exchange of bound GDP for GTP (or [^{35}S]GTPγS). This method has been used in purified and reconstituted systems [24], brain membranes [25], and cultured cell membranes [26, 27]. Cannabinoid receptor stimulation of [^{35}S]GTPγS binding has been quantified in brain membranes in the presence of excess GDP [68]. In rat cerebellar membranes, cannabinoid-stimulated [^{35}S]GTPγS binding is relatively high (2 to 3-fold stimulation), presumably because of the relatively large number of cannabinoid receptors in brain [7]. Moreover, this activation of G-proteins generally follows the pharmacology of the brain cannabinoid receptor binding profile, and is competitively blocked by the specific cannabinoid antagonist SR141716A.

The activation of [^{35}S]GTPγS binding by cannabinoid receptors has been adapted recently in our laboratory to localize receptor-mediated activation of G-proteins by *in vitro* autoradiography [28]. These studies have shown that cannabinoid activation of [^{35}S]GTPγS binding has a distribution similar to that observed for traditional cannabinoid receptor radioligand binding. For example, Fig. 2 shows a horizontal section of rat brain, comparing cannabinoid receptor binding and cannabinoid-stimulated [^{35}S]GTPγS binding, and demonstrates the high level of receptors and receptor-activated G-proteins in globus pallidus, hippocampus, and striatum. Interesting differences between the two systems are seen in several areas, e.g. relatively little G-protein activation (compared to receptor binding) is seen in thalamus, while relatively little receptor binding (compared to G-protein activation) is seen in cortex. These differences suggest that some brain regions may be more catalytically active than others. In fact, comparisons of Scatchard analysis for cannabinoid receptors and cannabinoid-stimulated [^{35}S]GTPγS binding revealed that cannabinoid receptors are not particularly catalytically efficient compared with other G-protein-coupled receptors. For example, each mu or delta opioid receptor in striatal membranes couples to approximately twenty G-proteins, while each cannabinoid receptor couples to only three G-proteins in the same region [29]. But the most important point about these experiments is that there is no region that contains appreciable cannabinoid receptor binding without appreciable cannabinoid-stimulated [^{35}S]GTPγS binding. Therefore, all cannabinoid receptors detected in brain with radioligand binding activate G-proteins. The biochemical identity of these G-proteins and the degree of efficiency of receptor-G-protein coupling are the subjects of current investigations.

Are the classical behavioral patterns elicited by THC correlated with this binding site? In humans, cannabinoid

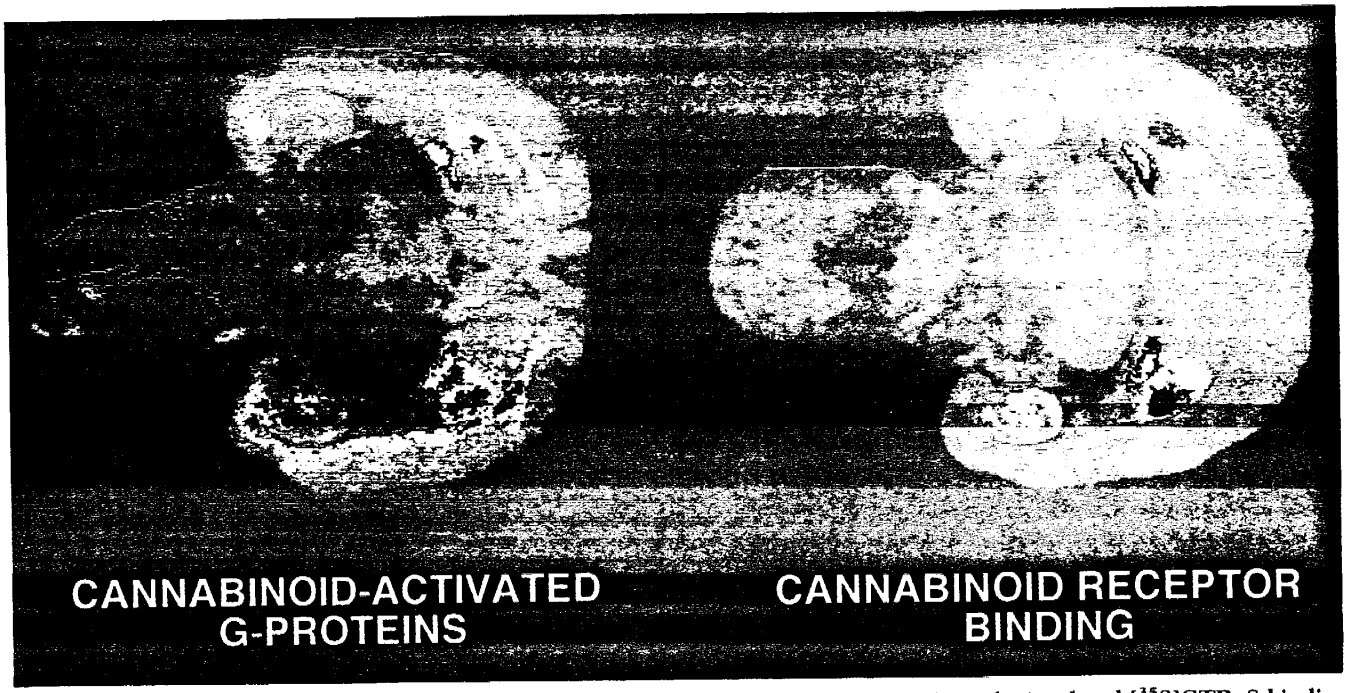

FIG. 2. *In vitro* autoradiography in a horizontal section of rat brain, comparing cannabinoid-stimulated [^{35}S]GTPγS binding (left), with WIN 55212-2 as an agonist, and cannabinoid receptor binding (right), using [^{3}H]WIN 55212-2.

effects are measured by psychoactive and motor activities [4]. In rats, the classical cannabinoid behavioral pattern is defined by a series of four behavioral tests; analgesia, hypomotility, lowered body temperature, and catatonia [30]. When affinities of cannabinoids in binding to brain receptors are compared to *in vivo* potencies in eliciting these behavioral patterns in rats, highly significant correlations have been observed. These correlations have not only been obtained from traditional cannabinoids and their analogs [30–33], but also for a series of anandamide analogs as well [34, 35]. The conclusions from all of these studies are that the structure–activity relationships of the binding studies correlate well with *in vivo* pharmacology, and that there is no reason to suggest that non-receptor-mediated mechanisms are responsible for any of the main behavioral effects of THC observed in rats. Whether such a correlation is true for humans, with psychoactivity as a behavioral end-point, is unknown.

EFFECTS OF CANNABINOIDS ON cAMP

Although cannabinoid-inhibited adenylyl cyclase is the best established of the signal transduction pathways for cannabinoid receptors, it is clear that other effectors are highly likely, and have been established in a number of different systems. For example, as mentioned above, receptors coupled to $G_{i/o}$-proteins modulate both calcium and potassium channels, and several groups have reported that cannabinoid agonists decrease Ca^{2+} conductance [36–39] and increase K^+ conductance through an inward-rectifying K^+ channel [40]. The effects on Ca^{2+} channels are not mediated by cAMP, but instead are likely directly coupled to G-proteins [36]. The involvement of cAMP in mediating a voltage-dependent K^+ channel ("A" current) will be discussed below. In addition to ion channels, other G-protein-coupled effectors have been associated with cannabinoid receptors; the most well known is the cannabinoid-induced release of arachidonic acid, observed in several cell culture systems and mediated by both phospholipase activity and G-proteins [41–43]. These findings illustrate the basic concept that G-protein-coupled receptors may operate through several different effectors, and each effector system may be designed for specific purposes.

Nevertheless, adenylyl cyclase remains a potentially important target for G-protein-coupled cannabinoid receptors, and it is necessary to review the characteristics of this signal transduction system. In virtually any cell that contains the necessary components of the ternary complex (cannabinoid receptors, G_i, and adenylyl cyclase catalytic units), cannabinoid agonists inhibit adenylyl cyclase. This has been demonstrated not only in cultured neuroblastoma cells [1], but also in cells transfected with both CB1 [14] and CB2 [44–46] receptors. Moreover, cannabinoid inhibition of cAMP levels has also been detected in non-transformed cells, and has been studied in mouse vas deferens [47] and rat cerebellar granule cells [48]. In addition, direct effects of cannabinoids on adenylyl cyclase activity have been determined in brain membranes [8, 49–51].

In general terms, the pharmacology of cannabinoid-inhibited adenylyl cyclase matches that of cannabinoid receptor binding [8]. However, precise correlations are not always easy to obtain, perhaps because the signal for this response is relatively low in brain membranes, and artifacts with the vehicles used to dissolve these lipophilic compounds are common. These technical difficulties may explain why a lack of correlation between cannabinoid-inhibited adenylyl cyclase and *in vivo* behavior has been reported [52]. The finding that the cannabinoid antagonist SR141716A acts as a competitive antagonist in blocking cannabinoid-inhibited adenylyl cyclase [45, 53] provides further evidence that this reaction is pharmacologically relevant.

In cerebellar granule cells, cannabinoid-inhibited adenylyl cyclase has been used to examine the relationship between the functional responses of cannabinoid receptors and other G-protein-coupled receptors. In addition to cannabinoid receptors [48], these cells also contain at least two other receptors coupled to inhibition of adenylyl cyclase: $GABA_B$ and adenosine A_1 receptors [54]. To examine whether these receptors shared common effector systems, both adenylyl cyclase and low K_m GTPase assays were performed using cannabinoid and $GABA_B$ agonists. Additivity experiments were performed where agonists of different receptor systems were added alone or in combination, to determine whether any portion of the signal transduction pathways is shared. Both of these receptors appeared to share common adenylyl cyclase catalytic units, since $GABA_B$ and cannabinoid agonists produced only a partial additive response for inhibition of adenylyl cyclase. However, $GABA_B$ and cannabinoid agonists produced a full additive response in stimulating GTPase [48]. The conclusion, as illustrated in Fig. 3, is that although different receptors may activate different pools of G-proteins, they may share common effector systems (in this case, adenylyl cyclase). An analogous situation has been observed with G-protein-linked receptors in hippocampal cells, where $GABA_B$ and 5-HT1a receptors share common K^+ channels [55]. This non-linear relationship between receptor, G-proteins, and effectors may explain why differences between chronic cannabinoid effects on receptor binding and on cannabinoid-inhibited adenylyl cyclase have been observed in cerebellar membranes from rats treated chronically with CP-55,940 [56].

ELECTROPHYSIOLOGICAL CONSEQUENCES OF CANNABINOID-INHIBITED ADENYLYL CYCLASE

If cannabinoid-inhibited adenylyl cyclase is prevalent in brain, what precise role does this second messenger system

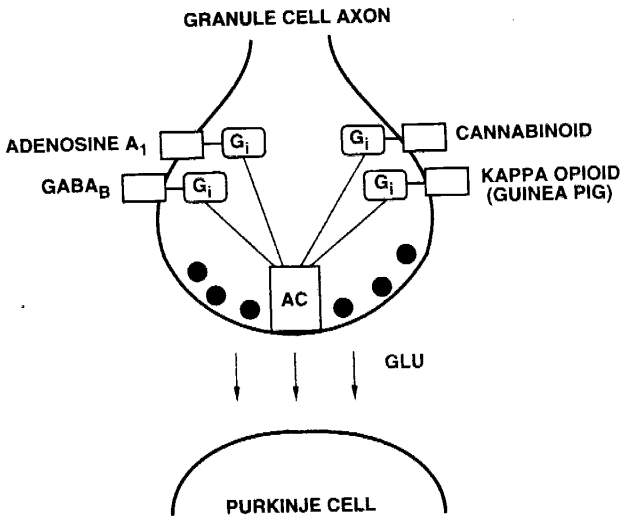

FIG. 3. Schematic diagram illustrating the presence of inhibitory G-protein receptors, including $GABA_B$, adenosine A_1, cannabinoid, and kappa opioid (in guinea pig) on cerebellar granule cell axons. These receptors are pharmacologically distinct and may couple to their own inhibitory G-proteins, but share common adenylyl cyclase (AC) catalytic units.

play in mediating the behavioral effects of THC? One system that has been well characterized is the cannabinoid-mediated alteration in the conductance properties of potassium channels, primarily those responsible for "A" current in primary cultures of hippocampal neurons [57]. Cannabinoids produce a positive shift in the voltage dependence of this channel; this process is cAMP dependent and results directly from cannabinoid receptor-mediated inhibition of adenylyl cyclase and subsequent inhibition of protein phosphorylation [58–60]. Several lines of evidence support this mechanism:

(1) The cannabinoid effect on potassium "A" current is mediated by $G_{i/o}$-proteins, since pertussis toxin eliminated, while non-hydrolyzable guanine nucleotide analogs mimicked, the receptor-mediated effect on potassium "A" current [57].

(2) The concentrations of cannabinoid agonists that modulated potassium "A" current in hippocampal cell cultures correlated with their potencies to increase low K_m GTPase in hippocampal slices [57] and with their potencies to inhibit adenylyl cyclase in brain membranes [49].

(3) The changes in "A" current produced by cannabinoid receptor activation were opposite those produced by treatments that elevated cAMP in the cell [59]. For example, the cAMP analogs 8-bromo cAMP and Sp-cAMPS, and the adenylyl cyclase activator forskolin, produce an effect in the opposite direction of cannabinoid receptor activation, whereas the cAMP blocking agent Rp-cAMPS produces an effect which is similar to that of cannabinoids [60]. The effect of forskolin, but not 8-bromo-cAMP, is reversed by activation of the cannabinoid receptor [61], consistent with the actions of an adenylyl cyclase-coupled receptor. Interestingly, procedures that increase cAMP levels in the cell produce a further negative shift in the voltage dependence of the potassium "A" current, indicating that the resting level of cAMP in these cells is between that inhibited by cannabinoids and that which could be further stimulated by cAMP analogs.

(4) Cannabinoid actions of potassium "A" currents are blocked by inhibitors of protein kinase A, including H-7, H-8, and HA1004 [60]. While it was only possible to inhibit 50% of protein phosphorylation with these inhibitors, this inhibition was sufficient to (1) block the cannabinoid-produced inhibition of cAMP and subsequent shift in voltage dependence of potassium "A" current, as well as (2) alter forskolin-stimulated increases in cAMP which also lead to shifts in the opposite direction of the voltage dependence of the potassium "A" current in the same cells. To investigate the next step in the cascade, IP-20, which inhibits the phosphorylation of the substrate protein by the catalytic subunit of the protein kinase, was administered [60]. This link confirmed the involvement of protein phosphorylation in the cAMP effect by showing that activation by the catalytic subunit of protein kinase A produced a shift in voltage dependence of the potassium "A" current. In all of the above cases, the appropriate manipulation either blocked or reversed the effects of the "upstream" activation by cannabinoids acting at the receptor. Figure 4 shows the complete cascade and what steps were probed with various techniques to prove linkage to cannabinoid receptor-mediated reduction in cAMP.

Two recent developments have provided further insight into this process. The selective antagonist SR17146A blocks all receptor-mediated effects of cannabinoids on potassium "A" current [62]. Surprisingly, however, addition of the antagonist alone, using concentrations slightly higher than those which block cannabinoid agonist actions, produces effects on "A" current which are similar to elevating cAMP. This suggests that either there is a population of "constitutively" active receptors whose blockade by the antagonist leads to reverse agonist effects, or there is a resting level of endogenous ligand (e.g., anandamide) whose binding to the receptor is blocked in the resting state. As stated above, the "resting tone" of the "A" current (i.e. the resting voltage dependence) is approximately midway between the extremes effected by maximum cannabinoid receptor occupancy or maximum elevation of cAMP levels by exposure to 8-bromo-cAMP [59]. The reverse agonist properties of the cannabinoid antagonist SR17146A, therefore, support the fact that resting receptor activated "tone" exists in the system, but provide no information about which mechanism is responsible for this effect. If this resting tone is produced by endogenous anandamide in the cell cultures, very large quantities of this ethanolamine would have to be

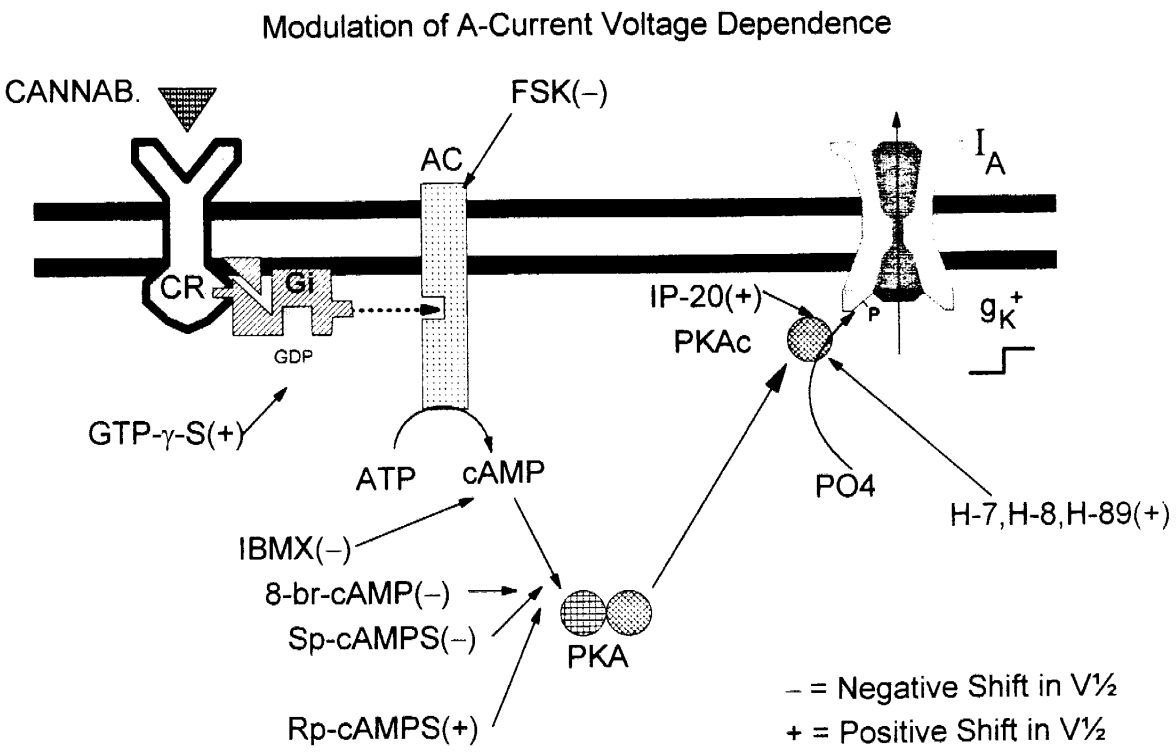

FIG. 4. Modulation of potassium "A" current (I_A) voltage dependence via cannabinoid inhibition of adenylyl cyclase. Pluses and minuses indicate positive and negative shifts in voltage dependence of I_A. In the absence of cannabinoids, cAMP from adenylyl cyclase (AC) stimulates cAMP-dependent protein kinase (PKA) and phosphorylates the I_A channel, resulting in a negative shift in voltage dependence of I_A. A cannabinoid agonist (Cannab.) binds to its receptor (CR) and inhibits AC through G_i proteins. The reduction in cAMP, and hence reduced phosphorylation of the I_A channel protein, results in a positive shift in I_A voltage dependence. Similar positive shifts are produced by the nonhydrolyzable GTP analog GTPγS, the inactive cAMP analog Rp-cAMPS, and PKA inhibitors IP-20, H-7, H-8, and H-89. Negative shifts are produced by cAMP analogs 8-Br-cAMP and Sp-cAMPS, the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX), and forskolin (FSK).

present, since the effects of the cannabinoid agonist on "A" current occur only at very high concentrations (50–100 μM) of anandamide (Mu J and Deadwyler S, unpublished observations).

OTHER TARGETS FOR cAMP

It is well known that CREs modulate gene expression by allowing cAMP-dependent protein phosphorylation to increase the synthesis of specific RNA [63]. Recent work in the opioid receptor field has demonstrated that not only can cAMP act as a positive promoter to increase gene expression, but also that receptor-mediated decrease in cAMP can attenuate RNA levels [64]. In this system, morphine was used as an agonist for mu opioid receptors to decrease forskolin-stimulated pro-enkephalin mRNA levels in rat striatum by decreasing forskolin-stimulated adenylyl cyclase. Current studies are now being performed to determine whether similar effects can be observed with cannabinoids. Such effects would indicate a role for cannabinoids in regulating long-term neuronal gene expression.

In addition, a number of intracellular enzymes are both calcium and cAMP dependent. An interesting connection to the function of cannabinoids was reported recently in the study of the synthesis of anandamide from membrane-bound precursors (*N*-acylphosphatidylethanolamine, or NAPE) [65]. In further studies [66], agents that increase cAMP levels (forskolin or vasoactive intestinal peptide) increased the synthesis of NAPE. Thus, levels of anandamide may be increased by increasing cAMP, whereas receptor-mediated inhibition of adenylyl cyclase may decrease anandamide synthesis. This latter mechanism would provide an effective negative feedback loop in which endogenous cannabinoids would inhibit their own synthesis. For example, increases in cAMP would lead to an increase in anandamide synthesis and, if subsequently "released," the anandamide could act back to reduce cAMP levels by binding to cannabinoid receptors. Consequently, if the release mechanism for anandamide was dependent on depolarizing events occurring in an axon terminal, the cannabinoid receptor-mediated reduction in cAMP would tend to inhibit release of anandamide by increased potassium "A" current hyperpolarization of the terminal after an action potential. This, in turn, would allow less anandamide to be available to bind to the cannabinoid receptor and hence decrease the inhibition of adenylate cyclase, thereby

causing cAMP levels to again rise and subsequently increase the synthesis of anandamide.

This has interesting implications for the role of cAMP in controlling the tendency of neurons containing cannabinoid receptors to respond under different conditions. In the presence of anandamide, neuron responsiveness would be decreased via enhancement in both potassium "A" current and reduction in calcium N channel conductances (not cAMP dependent). However, since the cAMP cascade is convergent from several other G-protein-coupled receptors on the same cell, any elevation in cAMP could eventually lead to an increase in the synthesis of anandamide [65, 66]. It becomes critical then to understand under what conditions anandamide is released and actually made available to cannabinoid receptors, since this will (1) result in inhibition of adenylate cyclase and reduction in cAMP in cells that possess those receptors, thereby decreasing synthesis of anandamide, and (2) reduce neural responsiveness to depolarizing agents in those same neurons. There are two locations in the cell where this could be strategically advantageous: (1) in the nerve terminal where such a cAMP-mediated shift in voltage dependence of potassium "A" current could limit depolarization-dependent release of all transmitters, and (2) in the dendrites and soma where spike frequency would be limited by increased potassium "A" current conductances triggered by action potentials in those locations [67]. In either case, the net effect of cannabinoid receptor occupancy would be to reduce transmission between neurons, and suppress activity within neurons.

CONCLUSIONS

Over 10 years after the identification of cannabinoid receptors, the precise nature of the signal transduction pathways responsible for the actions of THC is still not clear. However, several points can be made at this time:

(1) The G-protein-coupled cannabinoid receptors represent the principal mechanism of action of the cannabinoids. At this time, there is no compelling reason to propose that the main actions of these drugs are mediated by any non-receptor systems. A corollary to this point is that the first component of the cellular response of cannabinoids is the activation of G-proteins, although the precise identification of which G-proteins are activated is unclear at present.

(2) Many G-protein-coupled receptors, especially those which couple to $G_{i/o}$, modulate several different types of effectors, and the cannabinoid receptors are no exception. Thus, cannabinoid inhibition of adenylyl cyclase is only one of several signal transduction mechanisms for these receptors. Different effectors are useful under different conditions; for example, fast responses may be mediated by processes that are directly coupled to G-proteins, while slower responses are mediated through a diffusible cAMP/protein kinase system.

(3) cAMP is ideally situated to produce profound effects on intracellular signaling mechanisms for cannabinoids. As discussed above, the modulation of potassium "A" currents by a cAMP/protein kinase mechanism provides a crucial system for affecting hippocampal function. More long-term targets for cannabinoid-inhibited adenylyl cyclase may include inhibition of cAMP-dependent anandamide synthesis, and modulation of CRE to regulate neuronal gene expression. Many long-term effects of THC, including tolerance, may result from these kinds of mechanisms.

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