Pharmacology Biochemistry & Behavior, Vol. 40, pp. 565–569. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

The Cannabinoid Receptor: Biochemical and Cellular Properties in Neuroblastoma Cells

ALLYN C. HOWLETT, TRACY M. CHAMPION-DOROW, LORI L. McMAHON AND TRACY M. WESTLAKE

Department of Pharmacological and Physiological Science, St. Louis University Medical School
1402 South Grand Boulevard, St. Louis, MO 63104

HOWLETT, A. C., T. M. CHAMPION-DOROW, L. L. McMAHON AND T. M. WESTLAKE. *The cannabinoid receptor: Biochemical and cellular properties in neuroblastoma cells.* PHARMACOL BIOCHEM BEHAV **40**(3) 565–569, 1991.—The cannabinoid receptor that has been pharmacologically characterized for hypothermia, spontaneous activity, analgesia and catalepsy in rodents is the same pharmacological receptor that inhibits adenylate cyclase in vitro. The inhibition of adenylate cyclase by the cannabinoid receptor results from an interaction with G_i, based on the biochemical kinetic properties of the response, the sensitivity to pertussis toxin ADP-ribosylation, and the thermodynamic characteristics of the response. From precedents based on studies of the well-characterized G protein coupled receptors, rhodopsin and the β-adrenergic receptor, we can predict the tertiary structure of the cannabinoid receptor. Three sites of potential glycosylation are present on the receptor. However, treatment of N18TG2 neuroblastoma cells with tunicamycin to prevent glycosylation of newly synthesized receptors failed to alter cannabinoid-induced inhibition of cyclic AMP accumulation. The cannabinoid response was rapidly desensitized (within ½ h). Treatment of cells with tunicamycin failed to alter agonist-induced desensitization processes. These findings can be more veraciously interpreted as we gain a better understanding of the cellular dynamics of the cannabinoid receptor.

Adenylate cyclase Cannabinoid receptor Cyclic AMP Desensitization G protein Glycosylation
Neuroblastoma cells Tunicamycin

SUFFICIENT evidence has accumulated in recent years to support the contention that a number of behavioral responses to Δ^9-tetrahydrocannabinol (THC) and other CNS active cannabinoid drugs are attributable to neurons possessing cannabinoid receptors. Pharmacological characterization of hypothermia, spontaneous activity measures, analgesia, and catalepsy (hypokinesia) in rodents has demonstrated that these behaviors can be elicited in a dose dependent, pharmacologically specific and enantioselective manner by cannabinoid compounds (25, 28, 30). The parallel potency ratios for cannabinoid compounds would suggest that regulation of this quartet of behaviors is mediated by a common pharmacological receptor. Cannabinoid receptors found in abundance within cortical, hippocampal and basal ganglial structures of the brain (1,14) may be responsible for certain behaviors. Possible anatomical correlates of responses mediated by cannabinoid receptors in the CNS have been discussed in a recent review (22).

At the cellular level, CNS active cannabinoid compounds regulate the cyclic AMP second messenger system by binding to a membrane receptor and inhibiting adenylate cyclase. Cannabinoid compounds that are active in producing subjective and analgetic effects in humans, as well as in eliciting the quartet of behavioral responses in rodents, are able to inhibit adenylate cyclase in N18TG2 neuroblastoma membrane preparations with the same order of potency (16,20). For a series of cannabinoid agonists, the degree of enantioselectivity was related to potency for both in vivo and in vitro responses (21). Thus the receptor regulating the cyclic AMP second messenger system is pharmacologically identical to the cannabinoid receptor described in the behavioral studies. It should be noted that there exist numerous reports of actions of cannabinoid compounds, particularly those occurring at high concentrations of drug, for which the criteria of pharmacological specificity and enantioselectivity have not been met. The problems in the interpretation of these actions have been cogently discussed in a review of the cellular actions of cannabinoid drugs by B. R. Martin (29). It is likely that such actions are not mediated by the cannabinoid receptor that has been described for the above cited quartet of behavioral responses.

The present communication will describe the biochemical properties of the cannabinoid receptor and the second messenger response. Our current investigations of the cellular regulation of the cannabinoid receptor will be discussed with respect to the structural features of the protein.

The Cannabinoid Receptor: A G Protein-Coupled Receptor

The cannabinoid receptor is a member of the G protein-linked receptor family coupled to G_i to inhibit adenylate cyclase. Using purified membranes from cultured neuroblastoma cells, it was possible to demonstrate that cannabimimetic compounds cause a decrease in V_{max} of the enzyme with no alteration in K_m for the substrate or K_{act} for stimulatory neuromodulators, as expected for neuromodulators which regulate the enzyme via G_i (16,17). The cannabinoid regulation of adenylate cyclase is sensitive to divalent cations and guanine nucleotides in a manner characteristic of other G_i-linked receptors such as the muscarinic M_2 re-

ceptor. The inhibition was greatest at micromolar concentrations of Mg^{2+} or Mn^{2+}, and was abolished at ≥ 1 mM $MnCl_2$ for both muscarinic and cannabinoid inhibition. The concentration of GTP required for half-maximal inhibition of adenylate cyclase was 800 nM for both muscarinic and cannabinoid inhibition, whereas only 50 nM GTP was necessary for half-maximal stimulation by neuromodulators acting through G_s (17). The sensitivity to inactivation by low concentrations of Mn^{2+} and the requirement for relativity high GTP concentrations are attributes of receptor-regulated G_i mechanisms. The influence of a G protein on the cannabinoid receptor's agonist binding properties is also evident. Gpp (NH)p, a nonhydrolyzable GTP analog, caused a more rapid dissociation of the agonist ligand [^{3}H]CP-55,940 from the cannabinoid receptor, as expected for G protein-coupled receptors (9). These and other data argue against an action of cannabinoid compounds at the level of the adenylate cyclase catalytic protein or its regulation by stimulatory receptors via G_s. Rather, the demonstration that the biochemical properties of cannabinoid receptor binding and inhibition of adenylate cyclase are identical to those of the muscarinic receptor strongly argues that the cannabinoid receptor likewise interacts with G_i.

G_i can be functionally inactivated as a result of a covalent ADP-ribosylation catalyzed by pertussis toxin. We demonstrated that pertussis toxin treatment of neuroblastoma cells abolishes the cannabinoid response in intact cells and in membranes derived therefrom, confirming that G_i mediates the cannabinoid regulation of adenylate cyclase in the neuroblastoma model system (19). Later experiments showed that cannabinoid inhibition of cyclic AMP accumulation in rat brain slice preparations is significantly attenuated by intracranial microinjection of pertussis toxin (1). This finding is consistent with G_i mediating the cannabinoid response in mammalian brain as well as in the cultured neuronal cells.

The demonstration that CNS active cannabinoid drugs regulate adenylate cyclase only in certain neuronal cell types strongly argued for genotypic selectivity and against an involvement of plasma membrane perturbation in this response (8,19). Thermodynamic evidence to dismiss a role for perturbation of membrane fluidity as a mechanism for the inhibition of adenylate cyclase by Δ^9-THC was provided by studies of enzymatic activity as a function of temperature (18). A break point in the Arrhenius plot of hormone-stimulated adenylate cyclase occurs at 20°C, indicating the temperature at which the enzyme is sensitive to the gel-liquid crystal transition of membrane lipids. This transition temperature is reduced in the presence of a membrane fluidizing agent such as benzyl alcohol, which decreases the temperature at which the membrane lipids undergo a change in state. Δ^9-THC failed to alter the transition temperature even at concentrations that were 30-fold greater than required for maximal inhibition of adenylate cyclase. Analysis of the thermodynamic parameters indicated that Δ^9-THC inhibits adenylate cyclase by a mechanism whereby the entropy-driven stimulation of the enzyme by G_s is precluded. This mechanism is consistent with our current understanding of the regulation of adenylate cyclase by G_i.

Structural Features of G Protein-Coupled Receptors

Our current understanding of the structure and function of G protein-linked receptors is derived largely from studies of the β-adrenergic receptor coupled to G_s and rhodopsin coupled to transducin which have been summarized by Strader, Sigal and Dixon (36), and Wessling-Resnick and colleagues (37). These proteins were the first from this superfamily of G protein-coupled receptors to be purified to homogeneity and found to be transmembrane glycoproteins. Using cDNA probes to isolate

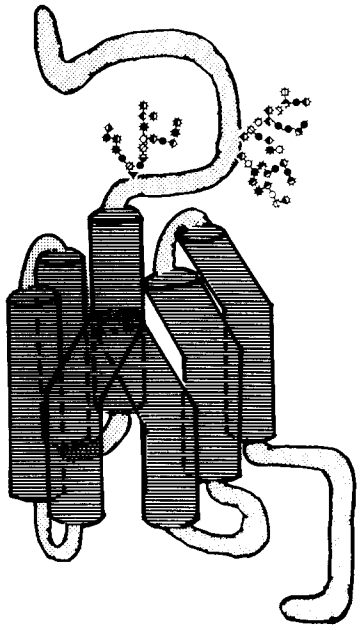

FIG. 1. Predicted tertiary structure of the cannabinoid receptor. The primary structure of the cannabinoid receptor deduced from the cDNA nucleotide sequence (31) comprises seven relatively hydrophobic domains (depicted as slashed cylinders) which would extend through the plasma membrane. The N-terminal extracellular (above) and C-terminal intracellular (below) extensions and intervening loops are depicted as stippled regions. In this crude representation, potential sites of glycosylation at asparagines 78, 84 and 113 on the extracellular extension (31) are depicted as branching patterns of heterogeneous sugar residues.

mRNA specific for these receptors, the entire amino acid sequences have been deduced. The secondary and tertiary structure has been predicted using hydropathicity analysis to determine regions of relative hydrophobicity that would be likely to form α-helical structures conducive to insertion within the lipid membrane. The G protein-coupled receptors exhibit a common basic structure of seven transmembrane-spanning regions with intervening loops extending intra- and extracellularly. Based on the tertiary structure determined by the electron diffraction pattern of bacteriorhodopsin [see review by Khorana (26)], the membrane-spanning regions for this family of receptors are believed to associate in a circular array to form a pore into which the ligand can fit (see Fig. 1). Confirmation of a similar structure for the β-adrenergic receptor has come from site-directed mutagenesis studies which have identified specific amino acids within a pore-shaped region critical for bonding with functional groups on catecholamines (36).

Using an oligonucleotide probe derived from a member of this G protein-linked receptor family, a cDNA clone for the cannabinoid receptor has been isolated from a rat cerebral cortex library (31). Evidence that this cDNA codes for the cannabinoid receptor came from studies that demonstrated the hybridization to mRNA in brain and in neuroblastoma cells known to possess cannabinoid receptors but not in a variety of other cells. Chinese hamster ovary cells that were stably transfected with the cDNA became responsive to cannabinoid drugs in assays for cyclic AMP accumulation, and this response was sensitive to pertussis toxin (31). Within the amino acid sequence deduced for the cannabinoid receptor can be found seven regions of relative hydrophobicity (31) that would be consistent with a seven trans-

membrane, pore-shaped tertiary structure. Thus the cannabinoid receptor exhibits the basic structural features predicted for a G protein-coupled receptor (Fig. 1).

Glycosylation and the Cannabinoid Receptor

The presence of high-mannose or complex oligosaccharide groups are a structural feature common to G protein-linked receptors. The extracellular N-terminal region of the β-adrenergic receptor exhibits asparagine residues within the appropriate consensus sequence (Asn-X-Ser/Thr) that are substrates for post-translational N-linked glycosylation (2, 6, 11, 12, 33, 35). Glycosylation has also been demonstrated for prostaglandin E_1 (12), histamine H_1 (32), muscarinic (15), δ-opioid (27), and dopamine D_2 (24) receptors. A consistent functional role for glycosylation within this family of receptors has yet to be established. For various receptors, the complex oligosaccharide groups have been implicated in the expression of high affinity binding (6,32), recovery of receptor numbers that had been reduced as a result of down-regulation (11), transit of newly synthesized receptors to the plasma membrane where the receptors can function (6), or the appropriate coupling to G-proteins (2). In other studies, no demonstrable function of glycosylation was evident (12,33).

The primary structure of the cannabinoid receptor contains the consensus sequence for three potential glycosylation sites on the N-terminal extracellular extension (31). Our laboratory has investigated the possibility of a functional role for glycosylation of the cannabinoid receptor. N-Linked glycosylation is a post-translational modification occurring as a series of enzymatic reactions beginning in the rough endoplasmic reticulum and continuing through the Golgi apparatus prior to the translocation of receptors to the plasma membrane [for review, see Hubbard and Ivatt (23)]. The sequence of reactions is initiated by the addition of dolichol phosphate to the Asn residue followed by the coupling of N-acetyl glucose to this site. The enzyme catalyzing this reaction is inhibited by tunicamycin, and therefore progress through the subsequent reactions synthesizing high-mannose or complex oligosaccharide chains cannot proceed in tunicamycin-treated cells.

We tested the hypothesis that glycosylation of the cannabinoid receptor is required for the inhibition of adenylate cyclase. Neuroblastoma cells were treated for 24 or 48 h with tunicamycin at concentrations reported to block glycosylation but not protein synthesis. Within 24 h of treatment, cells had withdrawn their neuritic projections, rounded up and become suspended in the media. This is evidence that the oligosaccharide-dependent cellular adherence to the surface had been disrupted by this treatment. The response to desacetyllevonantradol was tested using hormone-stimulated (secretin) conditions, or using conditions of direct activation of the catalytic protein by forskolin. Figure 2 shows that tunicamycin treatment failed to alter the cannabinoid inhibition of cyclic AMP accumulation in intact neuroblastoma cells. This finding could be interpreted to indicate that the oligosaccharide groups are not necessary for binding of the agonist to the receptor or the subsequent interaction with G_i. However, this negative result could also be explained by the presence of sufficient functional glycosylated receptors that remain available on the plasma membrane after tunicamycin treatment to maintain a maximal response. In the absence of data concerning the rates of cannabinoid receptor synthesis and degradation, and the fraction of receptors that must be depleted in order for the maximal response to be diminished (i.e., spare receptors), the hypothesis cannot be immediately rejected.

Within the family of G protein-linked receptors that interact with adenylate cyclase, the agonist-receptor interaction is not limited to activation of the intermediator G protein. The presence of the agonist also promotes the development of homologous desensitization of the response and ultimately the sequestration and/or down-regulation of receptors. These phenomenon and putative mechanisms are reviewed by Clark (7) and Sibley and colleagues (34). By desensitization, we mean that subsequent exposure to the agonist ligand produces a less effective (or ineffective) response than that observed initially. In the case of the stimulation of adenylate cyclase by the β-adrenergic receptor, this is a very rapid event occurring within minutes of exposure to the agonist. The studies of rhodopsin and the β-adrenergic receptor have described a mechanism for desensitization that involves phosphorylation by a receptor kinase which recognizes the receptor conformation conferred by the interaction with an agonist. Phosphorylation of critical serine or threonine residues on the third intracellular loop and/or the intracellular C-terminal extension facilitates the interaction of the receptor with a protein believed to preclude interaction with the G protein. This state can presumably be reversed by dephosphorylation catalyzed by a phosphatase.

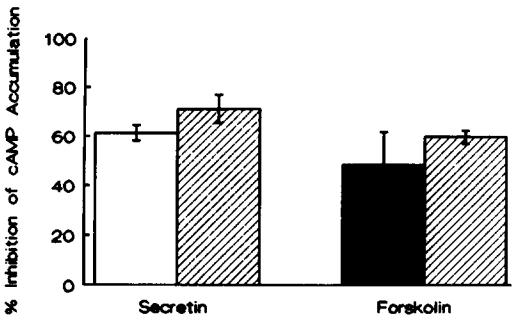

FIG. 2. Tunicamycin treatment does not block cannabinoid receptor inhibition of cyclic AMP accumulation. N18TG2 cells were seeded in 175 cm^2 flasks and allowed to attach to the surface for 24 h in media containing Ham's F12/Dulbecco's Modified Eagles (1/1) plus 10% donor calf serum and 2 μg/ml gentamycin as previously described (10). Tunicamycin (0.3 μg/ml) (Boehringer Mannheim) (hatched bars) or vehicle (NaOH) (solid bars) was added at 24 h and again at 48 h after plating. Cells were harvested at 72 h, washed, and resuspended in Gey's balanced salt solution containing 10 mM Hepes, pH 7.4 and 50 μM rolipram (Berlex Laboratories) as a phosphodiesterase inhibitor. Cyclic AMP accumulation was stimulated by 500 nM secretin (Bachem) or 3 μM forskolin (Calbiochem) in the absence or presence of 1 μM desacetyllevonantradol. After 4 min at 37°C, the reaction was stopped by the addition of 50 mM Na acetate, pH 4.5 and boiling. Cyclic AMP was determined by the procedure of Brostrom and Kon (5), and cellular protein was determined by the method of Bradford (4). The results are means ± SEM from N = 4 separate experiments.

Previous studies from our laboratory demonstrated that chronic exposure of N18TG2 neuroblastoma cells to Δ^9-THC or desacetyllevonantradol resulted in desensitization of the inhibition of cyclic AMP accumulation in response to cannabimimetic drugs (10). This desensitization was homologous because the response to carbachol via the muscarinic receptor was unaltered by previous exposure of the cells to desacetyllevonantradol. Exposure to either Δ^9-THC or desacetyllevonantradol at concentrations up to 100 μM failed to significantly affect cell growth rate, protein content or gross morphology at the light or the electron microscopic levels (10). The inhibition of cyclic AMP accumulation in response to desacetyllevonantradol could be attenuated by exposure to the drug for as little as one-half hour (Fig. 3). It may be hypothesized that in the initial phase of desensitization,

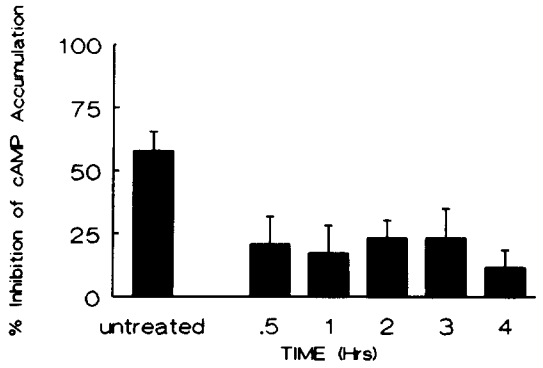

FIG. 3. Desensitization of the cannabinoid receptor inhibition of cyclic AMP accumulation occurs within ½ to 4 h of exposure to the agonist. N18TG2 cells were seeded at 5×10^5 cells/ml and allowed to attach as previously described in Fig. 2. After 24 h, media was changed to serum-free media (3). Desacetyllevonantradol (1 μM) or vehicle (fatty acid-deficient bovine serum albumin) was added, and cells were harvested at the indicated times. Cyclic AMP accumulation was determined in washed, resuspended cells as described in Fig. 2., except that 100 μM RO20-1724 (Roche) was used as a phosphodiesterase inhibitor, and secretin was used to stimulate adenylate cyclase. The results are means ± SEM from N = 4 separate experiments.

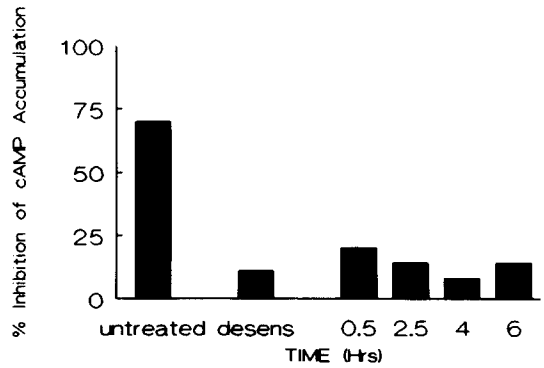

FIG. 4. Desensitization of the cannabinoid receptor inhibition of cyclic AMP accumulation is not recovered up to 6 h after removal of the agonist. N18TG2 cells were seeded and desensitized by addition of 1 μM desacetyllevonantradol or vehicle for 4 h as described in Fig. 3. At the indicated times, cells were harvested and washed, and cyclic AMP accumulation was determined as described in Fig. 3. The data are from a single experiment which was repeated with identical results.

a rapid modification may alter the coupling of receptors to G_i, consistent with a phosphorylation mechanism as described for other receptors of this family.

Down-regulation of receptors has been demonstrated for G protein-coupled receptors, however the mechanism is unclear. Over a time course of hours in the presence of the agonist, the number of β-adrenergic receptors measurable on the plasma membrane declines [for review, see (7,13)]. This has been shown to coincide with the appearance of receptors in an alternative membrane pool which is unable to interact with a G protein (sequestration). It is possible that a fraction of this pool of receptors may be recycled to the plasma membrane to resume functional interaction with G proteins. If these receptors become subsequently degraded (down-regulation), then the membrane receptors must be replaced by de novo synthesis. Similar cellular regulation may occur for the cannabinoid receptor. Following desensitization in N18TG2 cells, recovery of the cannabinoid response was not observed for at least 6 h after removal of the drug from the desensitized cells (Fig. 4). These data could suggest that continued exposure to cannabinoid agonists can result in a slowly reversible modification, such as a sequestration and possible degradation of receptors.

The hypothesis was tested that glycosylation of the cannabinoid receptor is necessary for the desensitization and subsequent responses initiated by the agonist-receptor interaction. Neuroblastoma cells were treated with tunicamycin for 24 to 48 h prior to initiation of desensitization by addition of the agonist for 24 h. The cells were harvested and the response to desacetyllevonantradol was determined. Figure 5 shows that tunicamycin treatment failed to modify the desensitization and/or down-regulation response using these standard test conditions. This could be interpreted to mean that the oligosaccharide groups are not necessary for the agonist-receptor interaction or subsequent events (e.g., phosphorylation and/or interaction with an inhibitory protein) that result in desensitization of the response or down-regulation of receptors. It is possible, however, that detailed concentration-response or kinetic analyses might define conditions that could reveal a sensitivity to tunicamycin treatment.

Summary

The N18TG2 neuroblastoma cell culture system has provided an ideal model to study the biochemical properties of the cannabinoid receptor-regulated cyclic AMP second messenger system. Using this model system, we were able to characterize a cannabinoid receptor which interacts with G_i and describe its biochemical properties. We can now study the cellular regulation of the cannabinoid receptor by pharmacological manipulation of the culture conditions. Initial studies have suggested that glycosylation of the cannabinoid receptor may not be important for agonist binding and subsequent interaction with G_i or development of desensitization. However, investigation of the rate of cannabinoid receptor synthesis and degradation are necessary to fully interpret these findings. Additionally, the role of phosphorylation of the cannabinoid receptor needs to be addressed. An understanding of the cellular regulation of the cannabinoid receptor should increase our understanding of cannabinoid actions in the brain, including the possible development of tolerance.

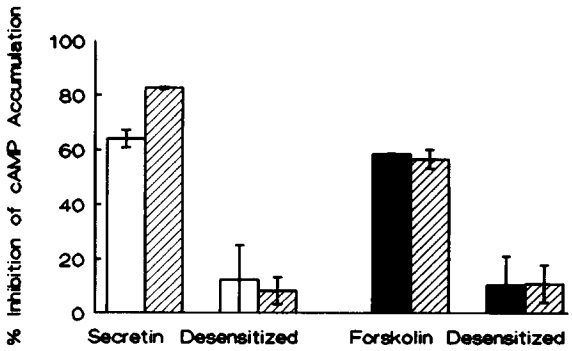

FIG. 5. Tunicamycin-treatment does not block desensitization of the cannabinoid receptor inhibition of cyclic AMP accumulation. Cells were maintained in culture as outlined in Fig. 2. Tunicamycin (0.3 μg/ml) (hatched bars) or vehicle (solid bars) was added for 24 h to 48 h. Desacetyllevonantradol (1 μM) or vehicle was then added for 24 h concurrently with tunicamycin. Cells were harvested at 72 h and cyclic AMP accumulation and protein content were determined as described in Fig. 2. The data are means ± SEM of data obtained from two to four separate experiments.

REFERENCES

1. Bidaut-Russell, M.; Devane, W. A.; Howlett, A. C. Cannabinoid receptors and the modulation of cyclic AMP accumulation in the rat brain. J. Neurochem. 55:21–26; 1990.
2. Boege, F.; Ward, M.; Jurss, R.; Hekman, M.; Helmreich, E. J. M. Role of glycosylation for β_2-adrenoceptor function in A431 cells. J. Biol. Chem. 263:9040–9049; 1988.
3. Bottenstein, J. E.; Sato, G. H. Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc. Natl. Acad. Sci. USA 76:514–517; 1979.
4. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
5. Brostrom, C. O.; Kon, C. An improved protein binding assay for cyclic AMP. Anal. Biochem. 58:459–468; 1974.
6. Cervantes-Olivier, P.; Delavier-Klutchko, C.; Durieu-Trautmann, O.; Kaveri, S.; Desmandril, M.; Strosberg, A. D. The β_2-adrenergic receptors of human epidermoid carcinoma cells bear two different types of oligosaccharides which influence expression on the cell surface. Biochem. J. 250:133–143; 1988.
7. Clark, R. B. Desensitization of hormonal stimuli coupled to regulation of cyclic AMP levels. Adv. Cyclic Nucleotide Prot. Phos. Res. 20:151–209; 1986.
8. Devane, W. A.; Spain, J. W.; Coscia, C. J.; Howlett, A. C. An assessment of the role of opioid receptors in the response to cannabimimetic drugs. J. Neurochem. 46:1929–1935; 1986.
9. Devane, W. A.; Dysarz, F. A., III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34:605–613; 1988.
10. Dill, J. A.; Howlett, A. C. Regulation of adenylate cyclase by chronic exposure to cannabimimetic drugs. J. Pharmacol. Exp. Ther. 244:1157–1163; 1988.
11. Doss, R. C.; Kramarcy, N. R.; Harden, T. K.; Perkins, J. P. Effects of tunicamycin on the expression of β-adrenergic receptors in human astrocytoma cells during growth and recovery from agonist-induced down-regulation. Mol. Pharmacol. 27:507–516; 1985.
12. George, S. T.; Ruoho, A. E.; Malbon, C. C. N-Glycosylation in expression and function of β-adrenergic receptors. J. Biol. Chem. 261:16559–16564; 1986.
13. Harden, T. K. Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. Pharmacol. Rev. 35:5–32; 1983.
14. Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S. De Costa, B. R.; Rice, K. C. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA 87:1932–1936; 1990.
15. Herron, G. S.; Schimerlik, M. I. Glycoprotein properties of the solubilized atrial muscarinic acetylcholine receptor. J. Neurochem. 41: 1414–1420; 1983.
16. Howlett, A. C.; Fleming, R. M. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol. Pharmacol. 26:532–538; 1984.
17. Howlett, A. C. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Mol. Pharmacol. 27:429–436; 1985.
18. Howlett, A. C.; Scott, D. K.; Wilken, G. H. The regulation of adenylate cyclase by cannabinoid drugs: Insights based on thermodynamic studies. Biochem. Pharmacol. 38:3297–3304; 1989.
19. Howlett, A. C.; Qualy, J. M.; Khachatrian, L. K. Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol. Pharmacol. 29:307–313; 1986.
20. Howlett, A. C. Cannabinoid inhibition of adenylate cyclase: Relative activities of constituents and metabolites of marihuana. Neuropharmacology 26:507–512; 1987.
21. Howlett, A. C.; Johnson, M. R.; Melvin, L. S.; Milne, G. M. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: Development of a cannabinoid receptor model. Mol. Pharmacol. 33: 297–302; 1988.
22. Howlett, A. C.; Bidaut-Russell, M.; Devane, W. A.; Melvin, L. S.; Johnson, M. R.; Herkenham, M.; The cannabinoid receptor: Biochemical, anatomical and behavioral characterization. Trends Neurosci. 13:420–423; 1990.
23. Hubbard, S. C.; Ivatt, R. J. Synthesis and processing of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 50:555–583; 1981.
24. Jarvie, K. R.; Niznik, H. B.; Seeman, P. Dopamine D_2 receptor binding subunits of M_r ~140,000 and 94,000 in brain: Deglycosylation yields a common unit of M_r ~44,000. Mol. Pharmacol. 34: 91–97; 1988.
25. Johnson, M. R.; Melvin, L. S. In: Mechoulam, R., ed. Cannabinoids as therapeutic agents. Boca Raton, FL: CRC Press; 1986: 121–145.
26. Khorana, H. G. Bacteriorhodopsin, a membrane protein that uses light to translocate protons. J. Biol. Chem. 263:7439–7442; 1988.
27. Klee, W. A.; Simonds, W. F.; Sweat, F. W.; Burke, T. R.; Jacobson, A. E.; Rice, K. C. Identification of M_r 58000 glycoprotein subunit of the opiate receptor. FEBS Lett. 150:125–128; 1982.
28. Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. J. Pharmacol. Exp. Ther. 247:1046–1051; 1988.
29. Martin, B. R. Cellular effects of cannabinoids. Pharmacol. Rev. 38:45–74; 1986.
30. Martin, B. R.; Balster, R. L.; Razdan, R. K.; Harris, L. S.; Dewey, W. L. Behavioral comparisons of the stereoisomers of tetrahydrocannabinols. Life Sci. 29:565–574; 1981.
31. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564; 1990.
32. Mitsuhashi, M.; Payan, D. G. Receptor glycosylation regulates the affinity of histamine H_1 receptors during smooth muscle cell differentiation. Mol. Pharmacol. 35:311–318; 1989.
33. Severne, Y.; Jurss, R.; Vauquelin, G. Deglycosylated mammalian β_2-adrenergic receptors are still able to undergo functional coupling to Ns. Biochem. Pharmacol. 35:4375–4380; 1986.
34. Sibley, D. R.; Benovic, J. L.; Caron, M. G.; Lefkowitz, R. J. Regulation of transmembrane signaling by receptor phosphorylation. Cell 48:913–922; 1987.
35. Stiles, G. L.; Benovic, J. L.; Caron, M. G.; Lefkowitz, R. J. Mammalian β-adrenergic receptors. J. Biol. Chem. 259:8655–8663; 1984.
36. Strader, C. D.; Sigal, I. S.; Dixon, R. A. F. Structural basis of β-adrenergic receptor function. FASEB J. 3:1825–1832; 1989.
37. Wessling-Resnick, M.; Kelleher, D. J.; Weiss, E. R.; Johnson, G. L. Enzymatic model for receptor activation of GTP-binding regulatory proteins. Trends Biochem. Sci. 12:473–477; 1987.