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Perspective

Neurotransmitter Receptor Binding and Drug Discovery

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Many psychotropic and cardiovascular drugs influence synaptic mechanisms to elicit their therapeutic effects. Until recently, drug discovery programs in these areas were not closely linked to basic research on neurotransmitter disposition, because molecular mechanisms of drug action had not been elucidated. Accordingly, in many cases one could not develop a systematic approach to identify new agents by monitoring in vitro biochemical actions.

Drugs can influence neurotransmitter systems in several ways. They may interfere with synthesizing or degrading enzymes, they may alter the storage or release of the transmitter, or they may mimic or block the actions of neurotransmitters at receptor sites. Many psychotropic and cardiovascular drugs act at receptors. Thus, to employ molecular strategies for drug development required simple biochemical assays for receptor recognition sites. With the exception of the nicotinic cholinergic receptor in electric organs of invertebrates, no neurotransmitter receptor could be monitored in simple binding paradigms applicable for drug screening until about 10 years ago.

Successful characterization of nicotinic cholinergic receptors in electric organs was dependent on the availability of extremely potent toxins, such as α-bungarotoxin labeled with ^{125}I, as well as the high density of nicotinic cholinergic receptors, almost 20% of membrane protein in the electric organ of *Torpedo marmorata*. By contrast, one could calculate that drug and neurotransmitter receptors, such as the opiate receptor, should constitute no more than about one-millionth by weight of brain or heart. It came as something of a surprise, then, that one could utilize simple reversibly binding drugs to label receptor sites in relatively crude preparations of membranes from brain and other tissues. This approach was first applied successfully with the opiate receptor.[1-4] Soon thereafter the same general strategy was used with appropriate ligands to label glycine,[5] muscarinic cholinergic,[6,7] GABA,[8,9] β-adrenergic,[10-12] dopamine,[13-15] and α-adrenergic receptors,[16-18] as well as receptors for a variety of peptide neurotransmitter candidates.[19] These simple, sensitive, and specific assays provided valuable screens to assess potencies of test chemicals.

One major issue in drug development is evaluating the extent to which an experimental substance is an agonist, antagonist, or mixed agonist–antagonist. In several drug classes, agents possessing particular ratios of agonist to antagonist activity may have unique efficacy. Thus, mixed agonist–antagonist opiates are often potent analgesics but with lesser addictive potential than pure agonists. Among β-adrenergic antagonists, agents possessing a certain amount of agonist properties, such as pindolol, may offer certain therapeutic advantages. In the first binding studies with the opiate receptor, it was not possible to distinguish between agonists and antagonists. Soon, we found that sodium ion dramatically distinguishes receptor interactions of agonists and antagonists, decreasing the affinity of agonists markedly, having no effect upon the potency of pure antagonists, and affecting mixed agonist–antagonist opiates in an intermediate fashion.[20] Subsequently, it was shown that guanine nucleotides, such as GTP, selectively decrease the affinity of opiate agonists and many other

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neurotransmitter agonists for receptor sites, presumably by binding first to the N-protein, which links receptors to adenylate cyclase, and allosterically altering receptor binding.[21,22] At opiate receptors, sodium and GTP appear to interact syngergistically so that monitoring the combined effects of sodium and GTP on drug–receptor interactions is the most powerful predictor of agonist–antagonist effects.[23]

Tissue selectivity of drugs is also of importance. One strategy for attaining such selectivity involves designing drugs specific for subtypes of receptors. The distinctions of muscarinic and nicotinic cholinergic receptors, of α- and β-adrenergic sites, and of β_1- and β_2-adrenergic receptors were established by classical pharmacological techniques. The advent of receptor-binding technology has enhanced the subtlety of receptor discrimination, confirming and extending distinctions suggested by traditional pharmacological approaches and indicating new receptor subclasses. Thus, binding studies have identified subclasses of opiate receptors, such as μ, δ, κ, and possibly σ.[24-26] Some opiate receptor subtypes, such as μ, κ, and σ were first proposed on pharmacological grounds with subsequent binding confirmation, while the μ–δ discrimination emerged first from binding work. At least two subtypes of dopamine receptors, D_1 and D_2, can be reliably discriminated by adenylate cyclase effects as well as binding techniques.[27-29] Several subtypes of muscarinic cholinergic receptors have been distinguished.[30,31] α_1-Adrenergic and α_2-adrenergic receptors can be discriminated,[32,33] and distinct high- and low-affinity subtypes of α_2 sites can be monitored.[34,35] Several classes of GABA receptors have been identified,[36] and benzodiazepine receptors, which are linked to GABA receptors, have been distinguished into at least three subtypes. Adenosine receptors can also be subdivided by binding and adenylate cyclase studies into at least two classes.

There are several ways in which drugs selected for one or another receptor subtype might offer unique therapeutic advantage. For certain drug classes, therapeutic effects might involve one receptor subtype and side effects another. There has been speculation that one subclass of benzodiazepine receptors might be responsible for the relief of anxiety, while another subtype elicits sedation. It has similarly been suggested that one subtype of opiate receptors, μ receptors, is primarily involved in the analgesic effects of opiates, while other receptor subtypes, such as δ receptors, may be involved in the influences of opiates upon mood.[37] The μ opiate receptors have been further subdivided into μ_1 sites, responsible for analgesia, and μ_2 receptors which mediate respiratory depression.[38] The enhanced therapeutic potential of μ_1-selective opiates is readily apparent.

Some receptor subtypes are differentially localized within the synapse. Thus, most α_1-adrenergic receptors are postsynaptic, while α_2 receptors are predominantly presynaptic, located on the terminals of norepinephrine neurons where they regulate norepinephrine release.[32] Antihypertensive drugs, such as prazosin, selectively block α_1 receptors and so do not elicit tachycardia as do drugs that block α_2 receptors on norepinephrine terminals, triggering excess norepinephrine release. In some and possibly all brain areas, benzodiazepine type I receptors appear to be postsynaptically localized, while type II sites are presynaptic.[39]

Some receptor subtypes may differentiate various tissues. Thus, the lungs are enriched in β_2-adrenergic receptors, while the heart has primarily β_1 types. Selective β_1 antagonists can provide therapeutic effects in cardiovascular disease without exacerbating asthmatic symptoms by blocking β_2 receptors in the lungs. Similarly, β_2 agonists can relieve asthma without causing tachycardia at β_1-adrenergic receptors in the heart.

Other advantages in drug development derive from the ability to measure drug activity in receptor-binding experiments. For most receptors one can screen up to a 100 chemicals in a day, whereas in vivo screens may require a day for only one or two drugs. Evaluating a drug in intact animals at several doses may consume 20–40 rats, while one can conduct several thousand binding assays on a single rat brain. Administering drugs to intact animals demands several grams of the test substance, while a milligram is generally more than ample for receptor screening.

Most importantly, receptor–binding techniques provide data that are substantially more valuable for systematic structure–activity analysis than can be obtained with in vivo studies. For instance, if four drugs in a series differ in potency in vivo, one cannot distinguish whether the differences are related to variations in drug metabolism, absorption from the gut, penetration to a target organ, or actual affinity for the receptor site. By contrast, in vitro studies provide precise molar affinities of drugs. This information can be fed back rapidly to the medicinal chemist, permitting further modifications in structure to provide a greater enhancement of potency. In this way one can amplify the potency of a test substance to a greater extent and more rapidly than with in vivo studies. For instance, over a period of 2 years a single postdoctoral fellow systematically modified xanthine structures, starting with theophylline and finally attaining a substance 70 000 times more potent than theophylline in blocking adenosine receptors.[40] Extremely high potency of a drug at its receptor site can provide selectivity of action, which makes for a lowered likelihood of side effects via actions at other sites. Once a substance has been sculpted for high receptor

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potency and selectivity, one can modify its structure, if necessary, to provide maximum bioavailability.

To show how neurotransmitter and drug receptors have been characterized and may be employed for drug discovery programs, it is useful to examine some receptors in detail.

Benzodiazepine Receptors. Using the same type of binding techniques that successfully identified opiate receptors, two groups independently described the binding of [^{3}H]diazepam to brain membranes.[41,42] The drug specificity of these binding sites fit well with the relative potencies of drugs as antianxiety agents in man and in animal behavioral tests.

Several features of the benzodiazepine receptor system offer opportunities for drug development. First of all, the ability to monitor potential therapeutic activity in simple binding tests enables researchers to screen for nonbenzodiazepine chemical structures that might have anxiolytic potential. Previously, initial screening for new therapeutic agents of this class required behavioral tests in animals. In most of such behavioral "conflict" models, the relative antianxiety potencies of drugs with benzodiazepine structures can be predicted with a substantial degree of precision. However, with unrelated structure, behavioral effects in animal tests may not relate directly to activity in humans. The ability to monitor binding to the specific therapeutic receptor site thus has greatly expanded the range of chemical substances that can be explored for antianxiety potential.

In the first studies of benzodiazepine receptor binding, numerous known neurotransmitters were screened for their ability to compete at receptor binding sites, but none were active. Drawing on the then quite recent identification of the enkephalins as endogenous ligands for the opiate receptor, researchers speculated that there might exist a novel "endogenous Valium" neurotransmitter.[41] Numerous laboratories attempted to identify such a substance but with no definitive success up to the present time. Of course, if an endogenous benzodiazepine-like neurotransmitter exists, it would represent a valuable model for new drug development.

The possibility that there is no specific benzodiazepine-like neurotransmitter became stronger when it was shown that GABA, the major inhibitory neurotransmitter in the brain, interacts physiologically with benzodiazepine receptors. Initial studies had employed high concentrations of GABA and looked for competition, hence, reduction of [^{3}H]diazepam binding. It was subsequently found that micromolar concentrations of GABA actually enhance [^{3}H]diazepam binding to receptors, an effect that becomes less evident with high GABA concentrations.[43-46] Various GABA-related amino acids mimic this action of GABA in proportion to their ability to mimic GABA synaptic activity. Moreover, specific GABA antagonists block the ability of GABA to enhance benzodiazepine receptor binding. Thus, the benzodiazepine receptor is, in fact, a site on a macromolecular complex that includes the GABA receptor.

It was not at first clear whether GABA and benzodiazepine receptors are completely distinct entities that are linked allosterically or whether they reflect a single protein complex. Observations that GABA stimulation of benzodiazepine binding persists after the receptors are solubilized[47] and even after extensive purification of the receptors[48] support the notion that the two receptors are part of the same protein complex. While benzodiazepine receptors have GABA recognition sites, heat protection experiments[49] and direct binding studies[50] indicate that GABA receptors also have benzodiazepine recognition sites.

The notion that GABA and benzodiazepine receptors are linked fits with an abundance of pharmacological data showing that benzodiazepines in low, pharmacologically active doses facilitate the synaptic actions of GABA.[51-53] Influences of GABA on benzodiazepine binding or vice versa appear to be molecular reflections of this synaptic synergism.

Further research has indicated other sites on the macromolecular GABA–benzodiazepine receptor complex. Barbiturates also stimulate benzodiazepine receptor binding.[54] However, they act at a site different than GABA, since the GABA antagonist bicuculline does not impair the effects of barbiturates. On the other hand, picrotoxin and related convulsants, which block chloride ion channels related to GABA synapses, antagonize the influence of barbiturates. GABA exerts its inhibitory synaptic activity by increasing the permeability of synaptic membranes to chloride ions. Thus, barbiturates might be influencing benzodiazepine receptor binding by acting at the chloride ion channel that is responsible for the synaptic actions of GABA. One can label this site in binding studies with [^{3}H]picrotoxinin[54] or the convulsant [^{35}S]TBPS (*tert*-butyl bicyclophosphorothionate).[55]

The apparent action of barbiturates at GABA-related chloride channels may be relevant for drug development. Barbiturates elicit physical dependence with chronic use and are lethal at relatively modest overdoses. Conceivably, novel structures acting at the barbiturate site might provide therapeutic actions with fewer of these adverse effects.

Mixed agonist–antagonists might have therapeutic utility at benzodiazepine receptors. Some relatively pure benzodiazepine antagonists bind with high affinity to benzodiazepine receptors but exhibit no benzodiazepine-like actions in behavioral tests.[56] However, they block the pharmacological effects of drugs such as diazepam. Whereas GABA increases the affinity of benzodiazepine agonists for their binding sites, it has no effect upon the receptor-binding affinity of pure benzodiazepine antagonists.[57,58] Drugs with mixed agonist–antagonist actions

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at benzodiazepine receptors should be affected in an intermediate fashion. Photoaffinity alkylation of benzodiazepine receptors with drugs such as flunitrazepam markedly decreases the potency of benzodiazepine agonists at receptors while producing no effect on receptor affinities of antagonists and intermediate actions on mixed agonist–antagonist.[59]

What pharmacological properties would one expect of a mixed agonist–antagonist at benzodiazepine receptors? Such drugs might produce therapeutic effects via their agonist component, while their antagonist properties would block the sedative potential. Drugs that display a mixed agonist–antagonist profile at benzodiazepine receptors have tended to fit this pharmacological profile. Clearly, screening drugs for mixed agonist–antagonist actions at benzodiazepine receptors may be a useful approach for drug discovery.

There appear to be several subtypes of benzodiazepine receptors. In the first studies with [^{3}H]diazepam, saturable binding sites were identified in peripheral tissues, such as the kidney, as well as in the brain.[41] However, some potent anxiolytics, such as clonazepam, which has nanomolar affinity for brain binding sites, displayed negligible activity in the kidney. On the other hand, Ro54864, a benzodiazepine that is not anxiolytic and has negligible potency at brain receptors, had nanomolar potency in the kidney. The physiological significance of the "peripheral type" benzodiazepine receptor is not yet clear. It can be readily labeled with [^{3}H]Ro54864.[60] The brain possesses some of these peripheral-type benzodiazepine receptors. However, whereas conventional central-type benzodiazepine receptors are localized in autoradiographic studies to highly selective synaptic areas of the brain, especially the limbic system, peripheral-type benzodiazepine receptors in autoradiographic studies are diffusely distributed throughout the brain. Indeed, after kainic acid destruction of neurons or in brains of patients with Huntington's disease in which glia proliferate, there is a marked enhancement in the numbers of peripheral-type benzodiazepine receptors in the brain.[61] Thus, for most of the brain, these receptors are not located on neurons but are associated primarily with glia.

In one part of the brain, the peripheral-type benzodiazepine receptors are associated with neuronal structures. Autoradiographic evaluations indicate a dense band of [^{3}H]Ro54864 binding in the glomerular layer of the olfactory bulb.[62,63] This is the layer of termination for the olfactory nerves that enter the brain from the nose. Destruction of olfactory nerves by irrigating the nose with zinc sulfate depletes [^{3}H]Ro54864 binding in the olfactory bulb.[63] Moreover, the nasal epithelium itself possesses extremely high levels of [^{3}H]Ro54864 binding sites, and autoradiographic studies indicate a localization of these receptors in the nose consistent with their occurrence on axons of olfactory nerves.[63] Thus, in the brain the peripheral-type benzodiazepine receptors are localized to olfactory nerves. What might be their function? If they were to influence the olfactory process, it might be possible to develop agents of therapeutic utility for absolute or relative anosmia, as occurs with chronic viral infections such as infectious mononucleosis and hepatitis. Since appetite is strongly dependent upon olfactory stimulation, agents that facilitate the sense of smell might be useful in treating anorexia associated with chronic debilitation in cardiac disease or cancer. The function of the peripheral-type benzodiazepine receptors in peripheral tissues, such as the kidney, is even less clear. Since diazepam itself has substantial potency at peripheral-type receptors, one might expect influences of diazepam upon renal function, though none have been reported.

Among the central benzodiazepine receptors there appear to be two subtypes. The first evidence came with the observation that the triazolopyridazine CL218872 was substantially more potent in inhibiting benzodiazepine receptor binding in the cerebellum than in the hippocampus.[64] It was suggested that the cerebellum was relatively enriched in a receptor subtype designated type I, which has selective high affinity for CL218872, while the hippocampus has a high proportion of type II receptors, which display less affinity for CL218872. Some carboline structures also display apparent type I receptor selectivity.[65] No type II selective drugs have yet been identified.

Evidence that type I and type II classes of binding reflect different receptor proteins comes from findings that the two receptor sites can be differentially solubilized from brain membranes.[66] Conventional detergents, such as Triton X-100, solubilize binding sites with a drug specificity of type II receptors. Type I receptors resist detergent solubilization however, but they can be solubilized by detergents combined with 1 M NaCl, and they retain their characteristic drug specificity when assayed in the soluble state.[66] There appears to be an anatomical basis for the differential detergent sensitivity of type I and type II receptors. Subcellular fractionation studies indicate that type I receptors are associated with postsynaptic densities which are well known to resist solubilization by detergents.[67] Thus, type I receptors have a postsynaptic localization. Type II benzodiazepine receptors appear to be located presynaptically, possibly on terminals of GABA pathways. In the substantia nigra, lesions of the descending striatonigral pathway, which includes a major GABA neuronal component, deplete type II receptors but increase the number of type I receptors.[39] This finding would fit with the ready solubilization of type II receptors by detergents, since nerve terminal membranes are much more readily solubilized by detergent treatment than postsynaptic structures. If the type II benzodiazepine receptors associated with descending striatal nigral neurons are in fact localized on GABA nerve terminals, they might regulate GABA release.

Is there therapeutic relevance for drugs selective at type I or type II benzodiazepine receptors? The type I selective drug CL218872 has an anxiolytic profile with minimal sedation, suggesting that type I receptors are responsible for relief of anxiety and type II sites mediate sedative effects.[64] On the other hand, type II receptors are most

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concentrated in limbic structures which have been thought to mediate antianxiety influences of drugs. Moreover, the relative antianxiety potencies of some drugs correlate better with their affinity for type II than type I receptors.[68] At the present time, the differential function of these two receptor subtypes is unclear.

Quite recently we have identified yet another site linked to benzodiazepine receptors. Drugs of the cyclopyrrolone class, such as suriclone and zopiclone, display benzodiazepine-like profiles in behavioral tests and compete for benzodiazepine receptor binding sites.[69] However, suriclone and zopiclone inhibit benzodiazepine binding in a noncompetitive fashion and alter the dissociation rate of [^{3}H]benzodiazepines.[70] Thus, suriclone and zopiclone may influence benzodiazepine binding by acting at a distinct site that is allosterically linked to the benzodiazepine recognition site. What might be the relevance of the suriclone–zopiclone site for drug development? In the limited clinical studies conducted up the present time, these drugs display therapeutic effects similar to those of conventional benzodiazepines. Conceivably, further clinical evaluation will disclose unique actions with potential therapeutic advantage.

Adenosine Receptors. Studies of adenosine are of interest for drug development, because adenosine exerts effects throughout the body so that one might seek drugs for numerous therapeutic applications. Thus, adenosine constricts the bronchi, and adenosine antagonists, such as theophylline, are bronchodilators. Adenosine dilates coronary blood vessels and may mediate coronary vasodilation following ischemia.[71] Xanthine adenosine antagonists are cardiotonics, while adenosine decreases cardiac contractility. Adenosine inhibits platelet aggregation, so that adenosine agonists might be of use in regulating blood clotting. In the brain, adenosine generally inhibits neuronal firing. Thus, adenosine antagonists would be anticipated to be stimulants.

The first biochemical approach to monitoring adenosine actions at receptors involved the measurement of cyclic AMP. Adenosine at nanomolar concentrations inhibits adenylate cyclase. This action is elicited quite potently by phenylisopropyladenosine (PIA) with stereospecificity such that L-PIA is substantially more potent than D-PIA.[72-74] Receptors mediating inhibition of adenylate cyclase by adenosine are referred to as A_1 receptors. In another nomenclature these are designated as Ri. At substantially higher micromolar concentrations, adenosine stimulates adenylate cyclase, an effect that is less stereoselective for isomers of PIA and is mediated by A_2 or Ra receptors.

To label adenosine receptors in binding studies, we developed [^{3}H]cyclohexyladenosine ([^{3}H]CHA),[75] while other groups independently utilized [^{3}H]PIA[76] or 2-chloro[^{3}H]adenosine.[77] Because of evidence that methylxanthines block adenosine actions on adenylate cyclase and numerous other systems, we synthesized 2,4-diethyl-8-phenyl[^{3}H]xanthine ([^{3}H]DPX) as a ligand.[75] Although adenosine receptors presumably occur in numerous tissues throughout the body, thus far specific binding has been demonstrated only in brain,[75] testes,[77,78] and fat cells.[76]

The drug specificity of [^{3}H]CHA, 2-chloro[^{3}H]adenosine, [^{3}H]PIA, and [^{3}H]DPX binding reflects predominantly A_1 receptor selectivity. *N*-Ethylcarboxamidoadenosine (NECA) displays A_2 receptor preference in influencing adenylate cyclase, and [^{3}H]NECA binding appears to involve A_2 as well as A_1 receptors.[79] With appropriate drug blanks, one can utilize [^{3}H]NECA to label A_2 receptors selectively (R. F. Bruns, personal communication).

How might adenosine receptor binding be utilized in drug discovery programs? The answer to this question depends on the physiological function of adenosine in various tissues. In the brain, adenosine seems to have presynaptic inhibitory actions, reducing the release of excitatory neurotransmitters. Direct evidence for this conclusion comes from autoradiographic studies showing the localization of adenosine receptors labeled with [^{3}H]-CHA to excitatory granule cell projections in the cerebellum and on excitatory optic nerve projections to the superior colliculus.[80] Since xanthines do block adenosine receptors, we wondered whether this effect can account for the stimulant actions of xanthines such as caffeine. Relative potencies of xanthines in blocking adenosine receptors correlate with their behavioral stimulant potencies, whereas their ability to block benzodiazepine receptors shows no such correlation.[81] PIA in low doses causes locomotor depression, which is dramatically reversed by xanthines.[81]

Evidence that the stimulant effects of xanthines involve adenosine receptor blockade suggests directions for novel therapeutic agents. Conceivably, stimulant actions of xanthine-like drugs might be useful in conditions such as exogenous, neurotic depression. The limitation of present xanthines derives from side effects such as tachycardia and diuresis, which might be overcome by centrally selective adenosine antagonists. Possible differences in drug specificity of central and peripheral adenosine receptors would facilitate design of such agents.

The behavioral depressant actions of PIA in animals is centrally mediated and not derived from peripheral hypotensive effects.[82] Rodents treated with PIA show decreased locomotor activity, but hypnotic effects are not observed even with considerably higher doses. This behavioral profile somewhat resembles that of benzodiazepines. Centrally selective adenosine agonists without peripheral hypotensive actions may thus have therapeutic potential.

It is not altogether clear whether bronchodilatory effects of known xanthines relate to adenosine receptor blockade, inhibition of phosphodiesterase, or neither. If these bronchodilatory effects can arise from adenosine blockade, then a drug exerting these actions without causing the tachycardia and central stimulation associated with theophylline would be useful.

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Attaining tissue selectivity for drugs depends in part on receptor heterogeneity. Besides the distinction of A_1 and A_2 adenosine receptors, we have found considerable species difference in drug specificity of A_1 receptors in brain tissue.[83] Thus, the xanthine DPX varies as much as 500-fold in potency at A_1 receptors in different species. If comparable differences in drug specificity for receptors can be identified in different tissues of a single species, tissue-specific drugs could be developed.

In principle, receptor-binding techniques should considerably facilitate the enhancement of drug potency by structure–activity analysis. For the xanthines, blockade of adenosine receptors can be readily augmented by structure–activity analysis. We developed a series of substituted xanthines with greatly enhanced potencies at adenosine receptors.[40] Thus, a 8-phenyl substituent augments the potency of theophylline about 1,000-fold. Replacing the 1,3-dimethyl substituents of theophylline with 1,3-dipropyl substituents produces yet a further 10-fold enhancement of potency. Varying substituents on the 8-phenyl ring also alters potency. A 2-amino-4-chloro substitution on the 8-phenyl group provides a 6-fold augmentation of potency compared to the unsubstituted 8-phenyl group. Combining all these substituents results in 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (PACPX), a compound which displays a K_i for adenosine A_1 receptors in bovine brain membranes of 22 pM. It is 4000000 more times potent than xanthine itself and 70000 times more potent than theophylline.

In summary, receptor-binding techniques provide powerful strategies for drug development in numerous therapeutic classes. The examples of benzodiazepine and adenosine receptors reviewed in detail here are only two instances. Comparable accomplishments should be feasible with all the known drug and neurotransmitter receptors.

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Communications to the Editor

Khellin Analogues. 1. General Topological Requirements for Lipid-Altering Activity in Furochromones

Sir:

The distribution of cholesterol among the various lipoproteins in the plasma is recognized as a powerful predictor of risk of cardiovascular disease and, in particular, of atherosclerosis.[1] Of the four classes of plasma lipoproteins, chylomicrons (specifically, chylomicron remnants),[2a] very low density lipoproteins (VLDL),[2b] and low density lipoproteins (LDL)[3-5] are considered atherogenic, whereas high density lipoproteins (HDL) have been reported to be antiatherogenic[6] (i.e., protective against atherosclerosis). The atherogenicity of LDL arises from increasing evidence that suggests that most cellular cholesterol is derived through the internalization and catabolism of LDL cholesterol by the cell.[7a] Since LDL is a product of VLDL catabolism,[7b] this latter lipoprotein is likewise regarded as being atherogenic. HDL cholesterol has been demonstrated to be a good predictor of coronary artery disease.[8] The inverse relationship between plasma levels of HDL and mortality from cardiovascular disease suggests that high levels of HDL may be protective against atherosclerosis,[9] and, in fact, considerable attention has been devoted to the metabolic control of HDL and factors affecting circulating HDL levels.[10] It remains to be seen whether manipulation of HDL levels will in itself alter the development of cardiovascular disease. A great deal of effort has gone into the identification of drugs that lower VLDL and LDL cholesterol and elevate HDL cholesterol, since such drugs should be antiatherosclerotic and, thus, provide a valuable therapy for reducing the risk of cardiovascular disease.[11]

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