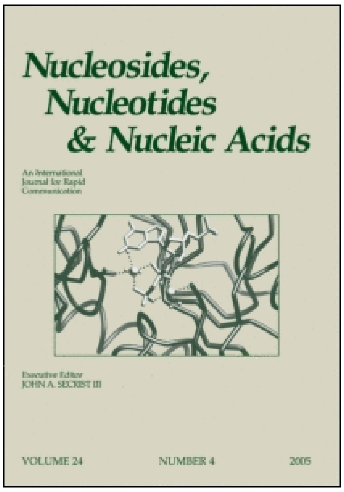


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Purine Functionalized Congeners as Molecular Probes for Adenosine Receptors

Kenneth A. Jacobson[a]; John W. Daly[a]
[a] Laboratory of Bioorganic Chemistry, National Inst. of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, MD

To cite this Article Jacobson, Kenneth A. and Daly, John W.(1991) 'Purine Functionalized Congeners as Molecular Probes for Adenosine Receptors', Nucleosides, Nucleotides and Nucleic Acids, 10: 5, 1029 — 1038
To link to this Article: DOI: 10.1080/07328319108047240
URL: http://dx.doi.org/10.1080/07328319108047240

NUCLEOSIDES & NUCLEOTIDES, 10(5), 1029-1038 (1991)

PURINE FUNCTIONALIZED CONGENERS AS MOLECULAR PROBES FOR ADENOSINE RECEPTORS.

Kenneth A. Jacobson* and John W. Daly

Laboratory of Bioorganic Chemistry, National Inst. of Diabetes, Digestive, and Kidney Diseases,
National Institutes of Health, Bethesda, MD 20892.

Abstract: The effects of distal structural changes on biological activity may be studied, using a "functionalized congener" strategy, in which suitably placed chemically reactive chains lead to biologically active conjugates. Probes for photoaffinity labeling, chemical affinity labeling, spectroscopic characterization and affinity chromatography of adenosine receptors have been derived from purine amine congeners (XAC, ADAC for A_1-receptors and APEC for A_2-receptors). Also, drug conjugates, including prodrugs and lipids, have been designed as potential pharmacological agents.

Classical development of congeners of biologically active agents has involved the alteration of specific sites in the pharmacophore (the essential portion of the drug molecule required for biological activity). This involves both substitutions with alkyl, aryl, heteroaryl, halogen, nitro, or sulfonamide moieties, and replacement of atoms, such as sulfur for oxygen. Such an approach often requires multi-step syntheses for each new congener and can be limited in scope because of the chemistry. In an alternate approach that is applicable to many classes of drugs, a starting point for new congeners is based on covalent attachment of chemically functionalized chains at insensitive sites (Figure 1) on the drug molecules. By this general "functionalized congener" strategy, the chain is modified in a stepwise fashion to optimize biological properties.[1-3] These functionalized drug congeners can be coupled covalently to diverse molecules, including large structures, resulting in a series of biologically active conjugates, each containing a common primary pharmacophore. Remarkably, distal structural changes can have marked effects on the biological activity.[1,3] The first effort to develop "functionalized congeners" targeted the ß-adrenergic receptor.[1, 29] A carboxy containing side chain replaced the isopropyl group of the potent ß-agonist isoproterenol. Various amides were prepared and a para-trifluoromethylanilide derivative had 215-fold greater affinity than isoproterenol at turkey erythrocyte receptors.[4] In a series of amino alkyl derivatives of the potent

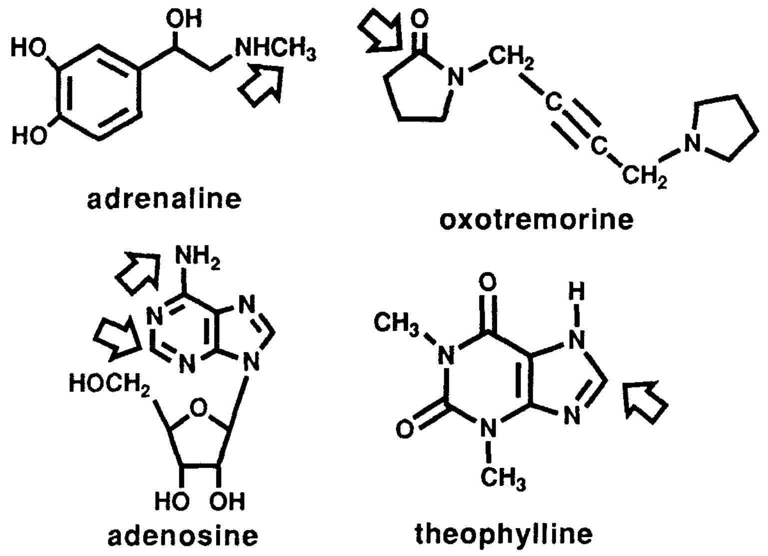


Fig. 1. Sites for introduction of *a functionalized side chain* into various pharmacophores.

muscarinic agonist oxotremorine, there was a chain-length dependent enhancement of muscarinic receptor affinity. However, unexpectedly the members of the series with the longest chains proved to be antagonists.[3] Some of the applications of the functionalized congener approach that can be developed are shown in Figure 2.

The functionalized congener approach has provided a wide range of potent, selective, and novel analogs[5-8] at A_1- and A_2-adenosine receptors. Functionalized congeners of adenosine, in which the derivatization site was the purine N^6- or C2-position, were found to be general A_1- and A_2-selective probes, respectively.[7,8] Alkylxanthines (the largest class of adenosine antagonists) likewise have been derivatized through the p-position of an 8-phenyl substituent.[2,5] Potency was defined initially by affinities for adenosine receptors in binding assays and in receptor-mediated effects on adenylate cyclase activity. Selected compounds have been screened in a variety of biological systems, including effects on the cardiovascular system[9] and on behavior.[10]

For both adenosine agonists and antagonists (Figure 3), the presence of a distal amino group enhanced potency. For example, the analogue of XAC in which the primary amino group was replaced by a hydroxy function was 8.5-fold less potent than XAC in the displacement of $[^3H]$R-PIA at A_1-adenosine receptors.[25] The purine amine congeners (XAC, ADAC, and APEC)

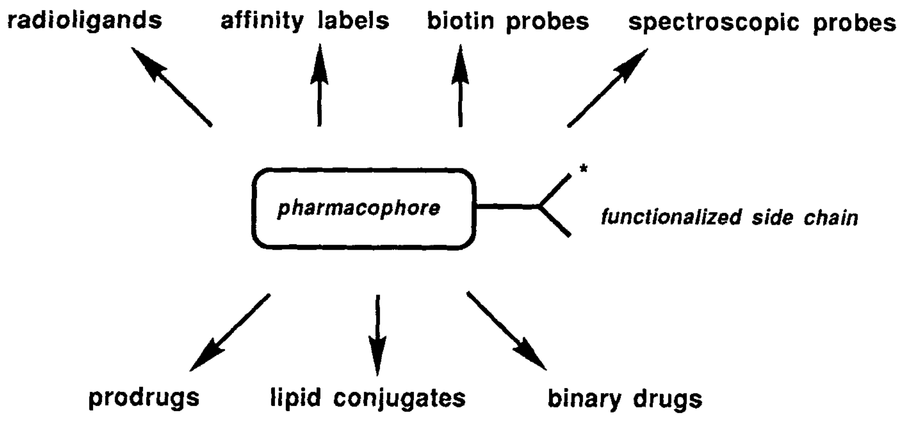


Fig. 2. Functionalized congener (* = COOH or NH_2) approach to receptor probes and novel drugs.

served as synthetic intermediates for the synthesis of probes for photoaffinity labeling,[11,12] chemical affinity labeling,[13] and affinity chromatography of adenosine receptors,[14,15] carried out in collaboration with Dr. Gary Stiles of Duke University Medical Center.

XAC (xanthine amine congener, antagonist),[5] ADAC (adenosine amine congener, A_1-agonist),[6] and APEC (2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5'-N-ethylcarboxamidoadenosine, A_2-agonist)[8,12] were designed as functionalized congeners: i.e., they contain a reactive functional group (an amine) that can be coupled covalently to other molecules resulting in further biologically active conjugates. APEC is a derivative of the A_2-selective agonist CGS21680.[26] XAC and ADAC are roughly 50-fold and 250-fold selective, respectively, for rat brain A_1-adenosine receptors, and APEC is roughly 40-fold selective for rat brain A_2-adenosine receptors. Conjugates of XAC, ADAC, and APEC have been prepared that contain prosthetic groups for the incorporation of radioisotopes, or contain fluorescent groups for spectrophotometric detection.[7] Such fluorescent groups include fluorescein, rhodamine, and nitrobenzoxadiazole. Purine functionalized congeners may be coupled to agarose affinity matrices or to bifunctional cross-linking reagents for irreversible receptor binding. Conjugates containing biotin to probe receptor topography, fatty acids to utilize lipid delivery, prodrugs, and binary drugs have been developed.

Receptor isolation

The primary amino group of XAC has been coupled to insoluble polymeric matrices, such as agarose, by Nakata[14] and by Olah et al[15] for the isolation of A_1-adenosine receptors by affinity chromatography. Biospecific elution of the receptor from the affinity gel was accomplished using

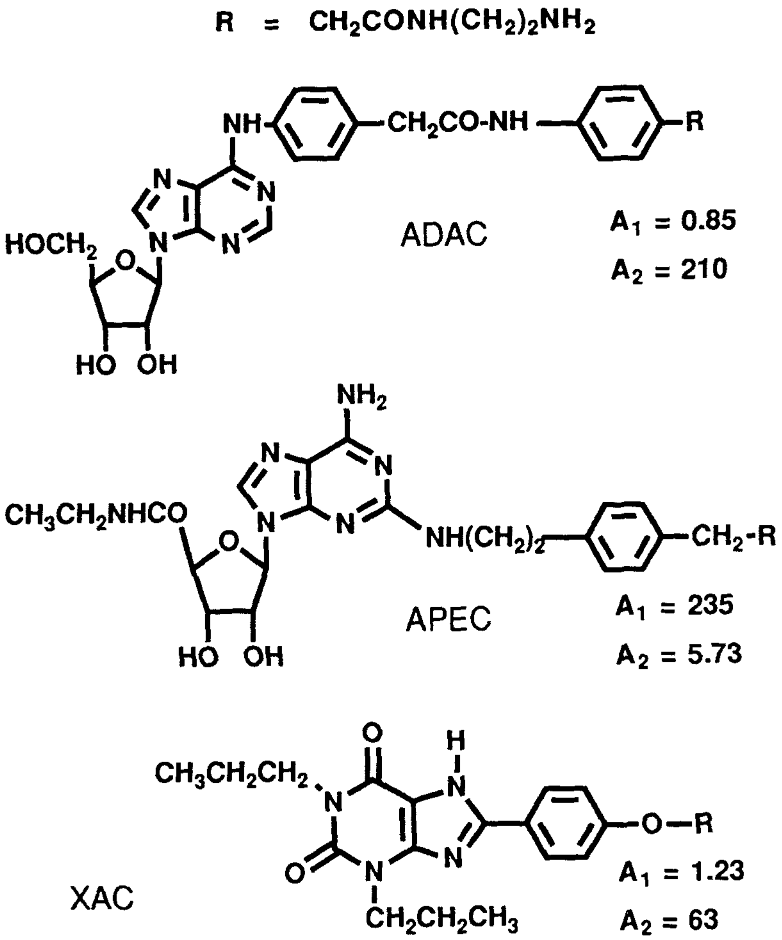


Fig. 3. Purine functionalized congeners (K_i values in nM, versus $[^3H]N^6$-phenylisopropyladensine at A_1-receptors or $[^3H]$ 5'-N-Et-carboxamidoadenosine at A_2-receptors in rat brain membranes).

either R-PIA or 8-cyclopentyltheophylline. The recovered bovine brain receptor, which was purified to homogeneity by Olah et al[16] using successive adsorption steps, was specifically labeled using the photoaffinity probe ^{125}I-PAPA-XAC (Figure 4) and shown to have a molecular weight of 38,000, in agreement with values previously determined for the receptor.

Photoaffinity labeling of A_1- and A_2-adenosine receptors

XAC and APEC were condensed to p-aminophenylacetic acid (PAPA) to form PAPA-XAC and PAPA-APEC, respectively (Figure 4). These aryl amines served as substrates for iodination with ^{125}I, for the labeling of A_1 and A_2-adenosine receptors, respectively.[11,12] The resulting radioligands bound to bovine brain adenosine receptors with a K_d of 0.1 nM (PAPA-XAC) and 1.5 nM (PAPA-APEC). The aryl amine was crosslinked to the receptor, either through photoaffinity crosslinking, using a photoactivatable azido (N_3) compound containing an active ester group (SANPAH) or by conversion of the amine to an azide.

Irreversible chemical labeling of A_1-adenosine receptors

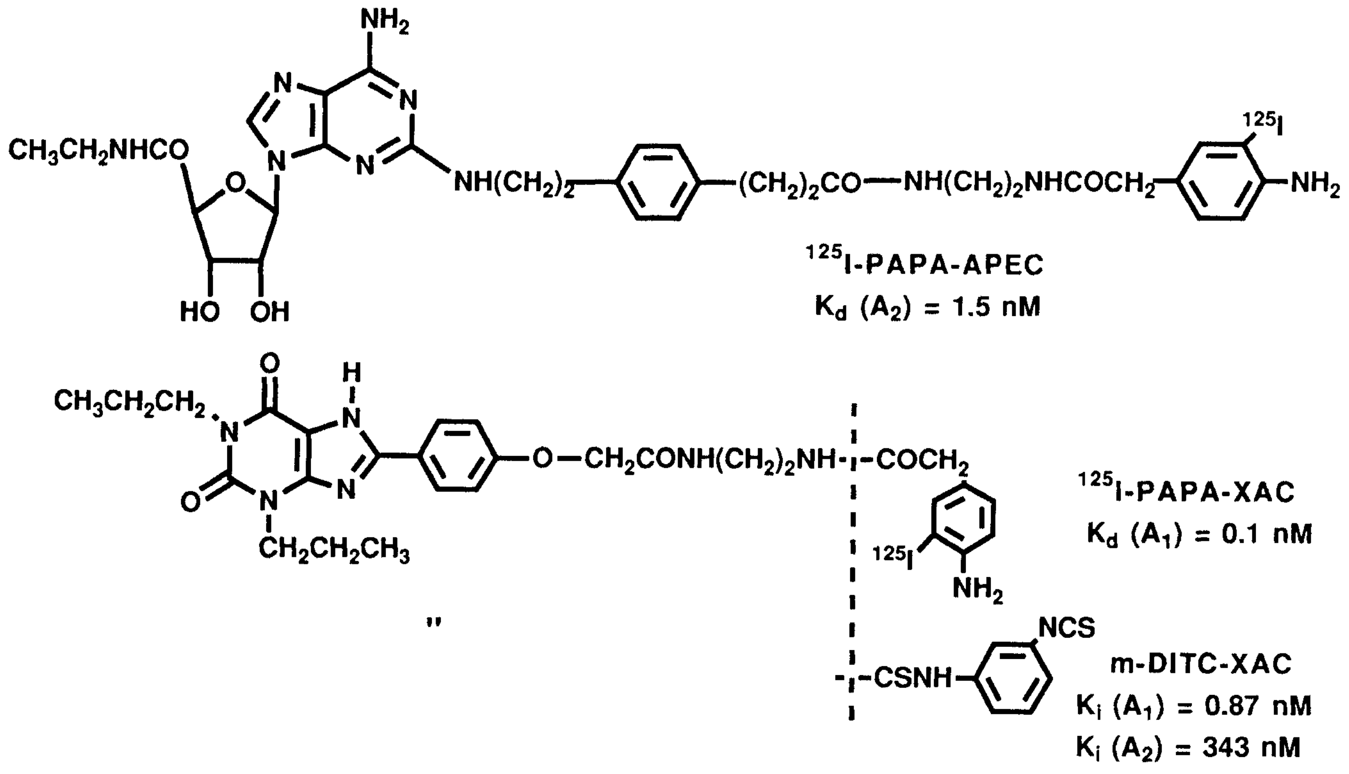
For chemical crosslinking to A_1 receptors, XAC was coupled to m- and p-phenylene diisothiocyanates.[13] The resulting conjugates, m- and p-DITC-XAC, contain a reactive isothiocyanate group that, through reaction with an amine or sulfhydryl group on the A_1-receptor, selectively inactivated the receptors during incubations of brain membranes with submicromolar concentrations of the xanthine. When the DITC-XAC isomers were synthesized using [^{3}H]XAC (a simple, single step reaction), the xanthine specifically labeled the receptor protein of molecular weight 38,000, as detected by SDS gel electrophoresis. We are now synthesizing highly water soluble (and thus useful for physiological studies) purine isothiocyanate analogs that will irreversibly inhibit adenosine receptors. Selective irreversible inhibitors of adenosine receptors may prove to be powerful physiologcal tools in the study of the regulatory effects of endogeous adenosine and the delineation of the role of A_1- and A_2- receptor subtypes.

Biotinylated probes

The avidin-biotin complex has been utilized in studies directed toward the isolation, histochemical localization, and microscopic structural probing of receptor proteins.[17] XAC, ADAC, and APEC have been coupled to biotin through spacer chains of varying length.[7,8,18] The resulting bifunctional probes were examined for the ability to serve as non-covalent cross-linkers between adenosine receptors and avidin. Affinity to A_1-receptors was measured in binding assays in the presence or absence of saturating avidin, under conditions in which it was shown that all of the biotin conjugate present was in the avidin-complexed form. Biotin derivatives of ADAC but not of XAC (Table 1) were capable of binding to both the A_1-adenosine receptor and to avidin concurrently.[18] This suggests topographical or conformational differences between the agonist-occupied and antagonist-occupied forms of the receptor.

Lipid conjugates

Another set of unconventional analogs that emphasizes the differences between receptor bound agonists and antagonists are long chain fatty acid amide derivatives of ADAC and



^{125}I-PAPA-APEC
K_d (A$_2$) = 1.5 nM

^{125}I-PAPA-XAC
K_d (A$_1$) = 0.1 nM

m-DITC-XAC
K_i (A$_1$) = 0.87 nM
K_i (A$_2$) = 343 nM

Figure 4. Structures of affinity labels for adenosine receptors. Affinity in nanomolar is expressed as K_d value in bovine brain or as K_i value in rat brain.

Table 1. Affinity of biotinyl-purine conjugates, alone and as complexes with avidin, at rat brain A1-adenosine receptors, K_i-values in nM versus [^{3}H]R-PIA in rat brain membranes, from refs. 7, 18).

Biotinyl (B-) derivative	Ki (no avidin)	Ki (avidin complex)
Biotinyl-ADAC	11.4	36
Biotinyl-ε-aminocaproyl-ADAC	18	35
ε-Biotinyl-D-Lys-ADAC	8.9	not determined
Biotinyl-XAC	54	>500
Biotinyl-ε-aminocaproyl-XAC	50	>500
Biotinyl-ε-aminocaproyl-gly$_3$-XAC	50	260

XAC.[25] Lipid conjugates have been used to alter the distribution or metabolism of drugs in vivo. In several cases, the lipid moiety has been cleaved enzymatically at a site of action to liberate an active molecular species.[27] Conceivably, a lipid conjugate of a drug could act at a receptor site prior to cleavage. As a means of possibly enhancing delivery of purines in vivo, fatty acids and other lipids were condensed to ADAC and XAC.[25] In binding assays at A_1-receptors, the ADAC conjugates displayed a dramatic enhancement of affinity as a smooth function of chain length of the lipid (Figure 5). In contrast, similar fatty acid amides and other lipid derivatives of the antagonist XAC did not display enhanced receptor affinity. The enhancement for ADAC conjugates was not affected appreciably by adding unsaturation to the fatty acid, and, thus, it was not markedly dependent on steric factors in the hydrophobic region. This suggested that the purines were "anchored" in a hydrophobic environment, presumably the phospholipid membrane bilayer. By analogy, other natural and synthetic receptor ligands that contain hydrocarbon chains, such as PAF-acether,[28] are thought to be stabilized in binding to receptors through a hydrophobic interaction with the surrounding biological membranes.

Prodrugs

The introduction of a reactive group in functionalized congeners, has made possible the development of a xanthine prodrug,[19] designed for selective activation in the kidneys. Theophylline, itself, has few sites for possible prodrug derivatization; indeed only derivatization at the N-7 imidazole nitrogen has been reported.[20] XAC, however, with a reactive alkyl amino group, is more readily derivatized for selective cleavage in vivo. The xanthine prodrug consisted of an amide derivative of XAC in which the primary amino group was condensed to the γ-carboxylate of glutamic acid. This design was based on a kidney prodrug scheme for amine-containing drugs first proposed by Orlowski, Wilk, and coworkers.[21] This scheme depended on the selective cleavage of γ-glutamyl amide derivatives by the enzyme γ-glutamyl transpeptidase (γ-GT), which

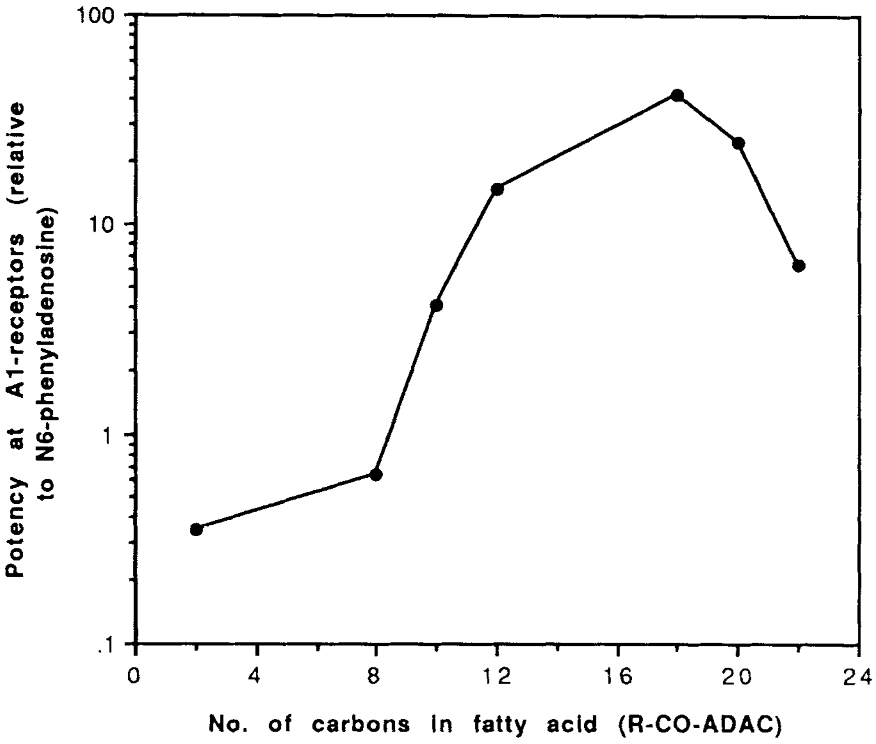


Figure 5. Receptor binding affinity of fatty acid amide derivatives of ADAC, versus [^{3}H]R-PIA in rat brain membranes (values from ref. 25).

is highly concentrated in kidney cells of the brush border membrane. The original prodrug scheme, as applied to sulfamethoxazole and dopamine, utilized a two-step cleavage process, in which an N-acyl-γ-glutamyl amide derivative of an amine-containing drug is cleaved first by an acylase, also concentrated in the kidneys, to give the γ-glutamide, and finally by γ-GT. Using two enzymatic cleavage steps to generate the potent drug resulted in greater specificity for the kidney than a single step cleavage dependent on γ-GT.

The beneficial therapeutic properties of adenosine antagonists in the kidneys, have been discussed.[22] XAC, like theophylline, is active as a diuretic and natriuretic agent, antagonizing the reduction of glomerular filtration rate induced by the adenosine analog, CHA.[19] At a dose of 5 mg/kg, administered in the tail vein in rats, XAC causes an increase in urine output that is comparable to that produced by theophylline. Thus, it would be desirable to achieve a selective *concentration in the* kidneys of an adenosine antagonist such as XAC, which is more potent and selective than theophylline.

γ-Glutamyl-XAC and its N-acyl derivatives were synthesized and explored as prodrugs for selective enzymatic cleavage in the kidneys.[19] In binding assays, the zwitterionic γ-Glu-XAC was a considerably less potent adenosine antagonist (K_i = 21 nM at rat A_1-receptors) than cationic amine derivatives, such as XAC and D-Lys-XAC (K_i = 0.87 nM), and thus would serve as a suitably masked, less active prodrug. The anionic N-acetyl-γ-glutamyl-XAC was even less potent (K_i = 298 nM). N-Acetyl-γ-glutamyl-XAC was not suitable as a prodrug for the two step cleavage process, as were the analogous derivatives in Orlowski's studies,[21] since it was not a substrate for purified renal acylase, an enzyme normally having broad specificity. Consistent with this biochemical observation, in vivo in rats N-acetyl-γ-glutamyl-XAC did not act as a diuretic, and free XAC was not observed in urine or in plasma. As predicted, γ-Glu-XAC did act as a diuretic, and was cleaved to XAC both in vivo and in vitro.

Binary Drugs

Purine functionalized congeners have been coupled to other biologically active pharmacophores, including substance-P peptides[23] and opioids,[24] to form "binary drugs," which were found to bind to either type of receptor as the covalent conjugate. The rationale for preparation of "binary drugs" is to enhance selectivity for receptors, tissues, or cell membranes that may contain both types of receptors, and to provide fro dual effects from a single binary drug.

Conclusions

The generality of the functionalized congener approach needs to be extended to the study of other membrane bound receptors. The potential for development of potent and selective agents and for molecular probes is amply illustrated at the adenosine receptors, while the generality of the approach is shown by the development of potent functionalized ligands for ß-adrenergic, muscarinic, and other receptors, that show marked structure activity relationships due to interactions at sites distal to the pharmacophore site.

REFEERENCES

1. Jacobson, K.A., Marr-Leisy, D., Rosenkranz, R.P., Verlander, M.S., Melmon, K.L., Goodman, M. *J. Med. Chem.* **1983**, 26:492-499.
2. Jacobson, K.A., Kirk, K.L., Padgett, W.L., Daly, J.W. *Mol. Pharmacol.* **1986**, 29:126-133.
3. Bradbury, B.J., Baumgold, J., Jacobson, K.A. *J. Med. Chem.*, **1990**, 33:741-748.
4. Schramm, M., Eimerl, S., Goodman, M., Verlander, M.S., Khan, M., and Melmon, K. *Biochem. Pharmacol.* **1986**, 35:2805-2809.
5. Jacobson, K.A., Kirk, K.L., Padgett, W.L., Daly, J.W. *J. Med. Chem.* **1985**, 28:1334-1340.
6. Jacobson, K.A., Kirk, K.L., Padgett, W.L., Daly, J.W. *J. Med. Chem.* **1985**, 28:1341-1346.
7. Jacobson, K.A., Ukena, D., Padgett, W., Kirk, K.L., Daly, J.W. *Biochem. Pharmacol.* **1987**, 36:1697-1707.
8. Jacobson, K.A., Barrington, W.W., Pannell, L.K., Jarvis, M.F., Ji, X.-D., Williams, M., Hutchison, A.J., Stiles, G.L. *J. Mol. Recognition*, **1990**, 2, 170-178.
9. Fredholm, B.B., Jacobson, K.A., Jonzon, B., Kirk, K.L., Li, Y.O., Daly, J.W. *J. Cardiovasc. Pharmacol.* **1987**, 9:396-400.
10. Nikodijevic, O., Daly, J.W., and Jacobson, K.A. *FEBS Letters*, **1990**, 261, 67-70.
11. Stiles, G.L., Jacobson, K.A. *Mol. Pharmacol.* **1987**, 32:184-188.
12. Barrington, W.W., Jacobson, K.A., Hutchison, A.J., Williams, M., Stiles, G.L. *Proc. Nat. Acad. Sci. USA* **1989**, 86:6572-6576.

13. Stiles, G.L., Jacobson, K.A. *Mol. Pharmacol.* **1988**, 34:724-728.
14. Nakata, H. *J. Biol. Chem.* **1989**, 264:16545-16551.
15. Olah, M.E., Jacobson, K.A., Stiles, G.L. *FEBS Letters*, **1989**, 257:292-296.
16. Olah, M.E., Jacobson, K.A., Stiles, G.L. *FASEB J.*, 4:A325, Abstract 354, Washington, DC, April, 1990.
17. Avidin-Biotin Technology, Methods in Enzymology, Vol. 184, M. Wilchek and E. Bayer, eds., Academic Press, San Diego, 1990.
18. Jacobson, K.A., Kirk, K.L., Padgett, W., Daly, J.W. *FEBS Letters* **1985**, 184:30-35.
19. Barone, S., Churchill, P.C., Jacobson, K.A. *J. Pharmacol. Exp. Ther.* **1989**, 250:79-85.
20. Bodor, N., Sloan, K.B., Kuo, Y.-N., Higuchi, T. *J. Pharm. Sci.* **1978**, 67:1045-1050.
21. Orlowski, M., Mizoguchi, H., Wilk, S. *J. Pharmacol. Exp. Ther.* **1980**, 212:167-172.
22. Churchill, P.C., Bidani, A.K. "Adenosine and renal function" in Adenosine and Adenosine Receptors, ed. M. Williams, Humana Press, **1990**, in press.
23. Jacobson, K.A., Lipkowski, A.W., Moody, T.W., Padgett, W., Pijl, E., Kirk, K.L., Daly, J.W. *J. Med. Chem.,* **1987**, 30:1529-1532.
24. Jacobson, K.A., Kirk, K.L., Mattson, M., Jacobson, A.E., Rice, K.C. 196th National Meeting of the American Chemical Society, Los Angeles, CA, Abstract MEDI87, Sept. 26, 1988.
25. Jacobson, K.A., Zimmet, J., Schulick, R., Barone, S., Daly, J.W., Kirk, K.L. *FEBS Letters*, **1987**, 225:97-102.
26. Jarvis, M.F., Schutz, R., Hutchison, A.J., Do, E., Sills, M.A., Williams, M. *J. Pharmacol. Exp. Ther.* **1989**, 251:888-893.
27. Garzon-Aburbeh, A., Poupaert, J.H., Claesen, M., Dumont, P. *J. Med. Chem.* **1986**, 29, 687-691.
28. Godfroid, J.J., Braquet, P., *Trends Pharm. Sci.* **1986**, 7:368-373.
29. Melmon, K.L., Khan, M.M. *Trends Pharm. Sci.* **1987**, 8:437-441.