Inhibition of Adenylyl Cyclase by Acyclic Nucleoside Phosphonate Antiviral Agents

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Acyclic derivatives of adenine, known as highly effective nucleotide analogs with broad spectrum antiviral activity, were evaluated for potential cross-reactivity with adenylyl cyclases, a family of membrane-bound enzymes that share putative topologies at their catalytic sites with oligonucleotide polymerases and reverse transcriptases. A series of derivatives of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) inhibited a preparation of adenylyl cyclase derived from rat brain with IC50 values that ranged from 66 μM (PMEA) to 175 μM for its diphosphate derivative (PMEApp) and mimics of it. PMEApp mimics included PMEAp(NH)p, PMEAp(CH2)p, PMEAp(CX)p (X = fluorine, chlorine, or bromine), PMEAp(CHX)p, and PMEAp(C(OH)CH3)p. The data suggest that inhibition of adenylyl cyclases may contribute to the therapeutic action of some of these or similar compounds or constitute part of their side effects in therapeutic settings.

Adenylyl cyclases are a family of membrane-bound enzymes central to one of the most important signal transduction systems and influence regulation of cell function in virtually all cells. The putative membrane topology of adenylyl cyclases conforms to a repeated sequence of a six-membrane spanning region followed by a cytosolic domain (1). The two cytosolic domains (C1 and C2) are homologous and contain regions that are highly conserved among adenylyl cyclase isozymes (1). The clef formed by interaction of C1 and C2 contains the enzyme catalytic site (2, 3), the topology of which resembles aspects of the palm domain of DNA polymerases and human immunodeficiency virus (HIV) reverse transcriptase (4–7). Moreover, aspects of the catalytic mechanisms of adenylyl cyclases and enzymes involved in polymerization of oligonucleotides are similar as well. Each involves nucleoside triphosphate as substrate and divalent cation-dependent catalysis that includes attack involving the substrate 3′-OH group, to catalyze either chain elongation of a primer oligonucleotide or formation of the 3′-5′-cyclic phosphate, with pyrophosphate as a leaving group. Adenine nucleoside 3′-polyphosphates are among the most potent inhibitors of adenylyl cyclases (8–11) and of these 2′-d-3′-ATP has been known for some time to bind to DNA polymerases (12).

Acyclic nucleoside phosphonates belong to a class of highly effective nucleotide analogs with broad spectrum antiviral activity (13–16). Of these 9-(2-phosphonylmethoxyethyl)adenine (PMEA) has demonstrated antiviral activity against HIV and different types of DNA viruses (13–16), differentiation-inducing activity (17, 18), and anti-tumor activity (18, 19). A related acyclic adenine derivative, PMPA, also is active against HIV and several members of the herpesvirus family (15, 20). Prodrug forms of these compounds provide transient protection of the phosphonate charge, allowing them to enter cells where they undergo deprotection and subsequent phosphorylation to their active inhibitory forms (19–23). Reported here is an evaluation of PMEA and various analogs of PMEApp as inhibitors of adenylyl cyclase.

EXPERIMENTAL PROCEDURES

Assay of Adenylyl Cyclase—Adenylyl cyclase was prepared as a detergent extract from rat brain as described previously (24, 25). IC50 values were determined graphically from logistic regression plots of adenylyl cyclase inhibition curves.

Materials—[α-32P]ATP was purchased from ICN Pharmaceuticals. 2′,5′-dideoxycytidine and the 3′-polyphosphates of 2′,5′-dideoxycytidine and of 2′-deoxycytidine were synthesized by methods previously reported (9–11). PMEA was prepared as described previously (21). The syntheses of the PMEApp mimics (Fig. 1) were developed from procedures of Moffitt (26), Blackburn et al. (27), and Holy and Rosenberg (28) and have been published in a preliminary communication (29). Lubrol-PX, used in experiments with the rat brain enzyme, was filtered through alumina (Neutral, AG7, from Bio-Rad) to remove peroxides.

RESULTS

The adenylyl cyclase extracted from rat brain has served as a basis for comparison of the effects of numerous adenine nucleosides and nucleoside phosphates (8–11). PMEA inhibited this enzyme with an IC50 of approximately 66 μM (Table I). Whereas this was notably higher than the previously reported inhibition by 3′-AMP or 2′-d-3′-AMP (IC50 8.9 and 12 μM, respectively), it was below that for 5′-AMP or 2′-d-5′-AMP (IC50 150 and >300 μM, respectively) (8), reflecting an intermediate capacity of this acyclic phosphonate to interact with this adenylyl cyclase. Because adenine nucleoside 3′-polyphosphates exhibited progressively lower IC50 values as 3′-phosphate groups were added (Table I) (9–11), and because it is the diphosphate derivative of PMEA that effects its antiviral action in intact cells (21, 30), the diphosphate form of PMEA was also tested. PMEApp and the corresponding imidodiphosphate derivative, PMEAp(NH)p, exhibited comparable potencies (IC50 ~170 and ~180 nM, respectively). Although PMEApp and
Adenylyl cyclases catalyze formation of cAMP from 5'ATP and also from 5'APP(NH)P and 5'APP(CH2)P but with reduced catalytic efficiency (24). The presumption has been that because of their changed electronic structures the imidodiphosphate and methylenediphosphate moieties do not form as good a leaving group as does inorganic pyrophosphate. Consequently, it was not surprising that the bg-methylene diphosphate derivative PMEAp(CH2)p (IC50; 5 mM) exhibited a reduced inhibitory potency compared with the unmodified PMEApp (Fig. 2 and Table I). Halogen substitutions within the bg-methylene diphosphate caused noticeable but modest improvements in potency (Fig. 3) as did the substitution of the –CH2– group with –C(CH3OH)– (Table I). The most potent of these was PMEAp(CF2)p (IC50; 1.5 μM; Fig. 3 and Table I).

The effects on the cyclase of these substitutions were as expected from the altered size and electronic character of the pyrophosphate leaving group in these PMEApp mimics.

DISCUSSION

The topology of the adenylyl cyclase catalytic site and aspects of its catalytic reaction resemble those of oligonucleotide polymerases and guanylyl cyclases (3, 4–7, 12, 31–33). These similarities in catalytic mechanism and structure among distinctly different enzyme families suggest that agents acting within the catalytic cleft of one may interact also with the other. This has been borne out in earlier studies with these enzyme families with some ligands and was extended here with the inhibition of adenylyl cyclases by a class of 9-substituted adenine acyclic phosphonate derivatives (Table I). PMEA, its diphosphate derivative, PMEApp, and mimics of PMEApp inhibited adenylyl cyclase with potencies (IC50 <200 nM) that were reminiscent of those of 2',5'-dd-3'-ATP (IC50 = 40 nM) (9, 10) and β-l-2',3'-dd-5'-ATP (IC50 = 24 nM; Kd 16 nM) (35). These latter two nucleotides are the most potent nucleoside triphosphate inhibitors of this enzyme, but inhibit by different mechanisms. Inhibition by 2',5'-dd-3'-ATP is of the post-transition state of the enzyme through a noncompetitive, dead-end mechanism (9–11), whereas that by β-l-2',3'-dd-5'-ATP is of the pretransition state and is competitive (35). This implies that the catalytically competent and the 3'-nucleotide-inhibited configurations of the enzyme differ.

In addition to adenylyl cyclases, PMEApp inhibits viral re-
verse transcriptases (13–16). It is presumably this mechanism through which the oral produg derivative of PMEA (adefovir dipivoxil) effects the therapeutic and potential inhibition of retroviruses and hepatitis B virus (19, 21, 30), but it is less certain that this is the means by which this drug also enhances differentiation and exhibits anti-tumor activity (17–19). This bis(S-acetyl-2-thioethyl) ester derivative of PMEA undergoes deprotection by cellular carboxyesterase(s) and subsequent phosphorylation to PMEApp to yield the active inhibitor of reverse transcriptases (21, 30). The data presented here, though, suggest that inhibition of adenylyl cyclase may contribute to either the therapeutic action or to the side effects of this drug. This caveat would also apply to analogously acting antiviral compounds in this class, such as PMPA (15, 18, 20, 23).

Given the central role that adenylyl cyclases play in the regulation of cell metabolism, function, and development, drugs that affect this enzyme family could be expected to elicit a variety of effects on cells. A pertinent example is the acceleration in differentiation in preadipocytes that was noted with adenine nucleoside inhibitors of adenylyl cyclase (34). Moreover, because there are at least nine adenylyl cyclase isozymes that are differentially expressed in cells and tissues, the effects of such drugs may also elicit tissue and cell-dependent effects.

Although this cross-reactivity of inhibitors likely extends to many of the enzymes for which nucleoside triphosphates are substrates and pyrophosphate is leaving group (3, 4–7, 12, 31–33), the identification of enzymes with which these antiviral agents interact and the knowledge of their structures and respective inhibitory profiles should allow more specific ligands to be designed. First, the similarity in catalytic mechanism and homologous structure among guanylyl and adenylyl cyclases (1, 31, 32) would suggest that guanine-based acyclic phosphonate derivatives would inhibit guanylyl cyclases and may be useful in reducing cellular cGMP levels. Second, if inhibition of adenylyl cyclase contributes to the side effects of the antiviral drugs, it can be readily designed around simply by use of some other base or by almost any modification of the adenine moiety, e.g. 3-deaza-, or 7-deaza-adenine (8–11, 35, 36). These changes would yield agents that do not affect adenylyl cyclase but which may yet inhibit oligonucleotide polymerases. Third, if therapeutic effects of adenine acyclic phosphonate derivatives are in fact brought about by inhibition of adenylyl cyclases or if inhibition of adenylyl cyclase yields the appropriate pharmacologic or therapeutic action, e.g. differentiation (34), more specific inhibitors could be developed that take advantage of our knowledge of the structures of this enzyme and its inhibitors. Some of the known potent inhibitors of this enzyme may be used or form the basis for the development of more selective ligands. The most effective agents might well be transition-state inhibitors, and adenine nucleoside 3′-polyphosphates are the closest to these of the known inhibitors of this enzyme family. Their usefulness for such purposes should be enhanced substantially by the use of transient protecting groups that will allow these compounds to enter cells and find application in intact cell systems. Other enzymes with nucleoside triphosphate as substrate and pyrophosphate as leaving group may be similarly and more specifically targeted.

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J. Biol. Chem. 1999, 274:34742-34744.
doi: 10.1074/jbc.274.49.34742

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