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Biochemistry

Use of a yeast expression system for the isolation and analysis of drug-resistant mutants of a mammalian phosphodiesterase

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ABSTRACT ***Saccharomyces cerevisiae*** **strain PP5 has a phosphodiesterase (PDE) deficiency that results in heat-shock sensitivity due to the intracellular accumulation of cAMP. This strain also carries the *cam* mutation, which confers permeability to cAMP and, as shown here, to other compounds. Expression of rat type IV PDE in these cells caused them to revert to heat-shock resistance. Treatment of the transformed PP5 cells with rolipram, an antidepressant in humans and a potent inhibitor of type IV PDEs, reinstated sensitivity to heat shock. The biochemical properties of deletion mutants of this PDE were determined, and an active enzyme of minimum length was created. Reversion to heat-shock resistance was then used to select for PDE mutants refractory to the inhibitory effects of rolipram. Four mutants (A1, A2, A3, and A5) were isolated. Each carries a single point mutation; two have mutations in the same codon. Each mutant showed distinct properties, based on analysis of their substrate kinetics and IC_{50} values for a variety of inhibitors. Mutant A5 had a reduced activity for substrate, mutants A1 and A3 showed no change in substrate kinetics, and mutant A2 displayed an increase in activity. For most mutants, the drug resistance was confined to the class of drug used in the selection. This study shows that it is possible to recreate in yeast cells the susceptibility of mammalian enzymes to pharmacological agents. Our study also demonstrates that such systems can be used to select rare mutants useful in the analysis of drug–protein interactions.**

Control of intracellular cAMP concentration is achieved through the regulation of cAMP synthesis by adenylyl cyclases and degradation by phosphodiesterases (PDEs). The mammalian PDEs form a particularly diverse family of isozymes. They have been categorized into seven types (1–3) based primarily on substrate selectivity and kinetics, response to activators and inhibitors and, more recently, primary sequence. PDEs of a given type, even across a wide evolutionary distance, can show extremely high and extensive conservation of sequence (4–7). Isozymes of different types, however, show only modest sequence conservation that is confined to a common catalytic domain (8, 9). Another important distinguishing characteristic of PDE isozymes is their tissue-specific expression patterns, which are overlapping and quite complex (10–15).

PDEs are the targets of a variety of pharmacological agents. Some compounds inhibit a wide spectrum of PDEs, whereas others are quite isozyme-specific (1, 16, 17). Given the wide range of pathologies in which cAMP plays a role and because compounds with the greatest PDE isozyme specificity should also display the highest therapeutic index, it is of interest to develop a rapid assay for isozyme-specific PDE inhibitors and to determine the nature of inhibitor–enzyme interactions.

We have described (4, 18) the isolation of mammalian PDE cDNAs by the use of a genetic selection in *Saccharomyces cerevisiae*. Yeast cells that carry the activated *RAS2* allele $RAS2^{val19}$ or that carry disruptions of both endogenous PDEs, *pde1 pde2*, are unable to undergo G_1 arrest in response to starvation conditions and, consequently, are heat-shock sensitive (4, 19). Expression of a mammalian PDE in these cells allows them to reduce intracellular cAMP levels and survive heat-shock treatment. We now report that the isozyme-specific drug rolipram can inhibit the activity of a mammalian type IV PDE in yeast cells. This inhibition can be readily assayed as drug-induced heat-shock sensitivity. We used this system (*i*) to characterize deletion mutants of a rat type IV PDE and (*ii*) to isolate point mutants refractory to rolipram. Analysis of several mutants has revealed the identity of residues required for drug interactions but not for enzyme activity. One mutant is affected in both drug and substrate interactions, a result consistent with a competitive mode of inhibition for this drug.

MATERIALS AND METHODS

Yeast and Bacterial Strains. Strain PP5 was derived from strain GC116-10A (*MATa leu2-3 leu2-112 ura3-52 his3-532 his4 cam*), which was provided by Warren Heideman. This strain was transformed with a *HIS4, URA3* plasmid (pB294), which was provided by Gerald Fink (Whitehead Institute for Biomedical Research, Cambridge, MA). These cells were then transformed with a *pde2::HIS3* disruption fragment (20). The pB294 could then be dropped, and these cells were transformed with a *pde1::URA3* disruption fragment (19). Disruption mutations were confirmed by Southern blotting. This strain, PP5 (*MATa leu2-3 leu2-112 ura3-52 his3-532 his4 cam pde1::URA3 pde2::HIS3*) was then tested to confirm heat-shock sensitivity.

Plasmids, Cloning, PCR, and Sequencing. The entire DPD cDNA insert of pADPD (4) was transferred into pUC118 by using *Hin*dIII and *Eco*RI to give pDPD. The unique *Hin*dIII site of pDPD was changed into a *Sal* I site by using the adapter oligonucleotide AGCTGTCGAC, giving pDPDS. Deletion mutants of DPD were created as follows. The carboxyl-terminal deletion was made by digestion of pDPDS with *Stu* I followed by ligation with the oligonucleotide CTAAGCTTAG, allowing the introduction of a stop codon and a *Hin*dIII site. Carboxyl-terminal deletions were created by replacing the *Sal* I–*Nsi* I fragment of pDPDS with PCR-generated fragments that deleted various amounts of sequence and introduced a *Sal* I site. Each PCR used the downstream primer CGTCTTTAGAAGGTCTCT. The upstream primers were CGATGTCGACCTTGCGAATCGTAAGAAA (Δ1), CGATGTCGACGCTGGAGGAACTAGA (Δ2), and CGATGTCGACTTCAAACAAGTTCAAAA (Δ3). PCRs were done by using standard reaction conditions. The Δ2 mutant,

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Abbreviations: PDE, phosphodiesterase; IBMX, isobutylmethylxanthine.

which served as the substrate for nitrosoguanidine mutagenesis, was sequenced in its entirety. Deletion constructs were moved first into pBluescript with *Hin*dIII and *Sal* I, and then they were transferred into pAD54N by using *Sal* I and *Not* I. pAD54N is a yeast expression vector similar to pADNS (4). pAD54N carries an influenza hemagglutinin epitope fusion-encoding sequence (20) immediately downstream of the promoter. The double mutants were created by exchanging the *Sac* I–*Not* I fragment from mutant A1 with mutants A2 and A3. Sequencing was performed by the dideoxynucleotide chain-termination method.

Heat-Shock Assays and Selection of Mutants. A library of mutant DPD clones was produced by treating ADPDΔ2-containing DH5α cells with nitrosoguanidine (21). PP5 cells were transformed with the library DNA and grown for 24–36 hr in liquid selective medium (SC-Leu) to maintain the plasmid. Cells were then transferred to yeast extract/peptone/dextrose (YPD) medium and allowed to grow for 3 days, after which 0.4 ml of culture was treated with 500 μM rolipram for 12–18 hr. Control cells were treated with ethanol (the rolipram solvent). The cultures were shocked at 52°C for 25 min in a water bath. Cells were plated onto YPD medium. Survivors were rechecked for heat-shock resistance. Heat-shock assays were done on agar plates (4). Yeast cells were transformed by using published protocols (22, 23).

Yeast Protein Extract Preparation and Immunoblot Analysis. Yeast cells were grown at 30°C for 15–24 hr in 15 ml of synthetic medium (SC-Leu) and then for 4 hr in yeast extract/peptone/dextrose (YPD). The cells were harvested, washed with buffer C (20 mM Mes/100 μM $MgCl_2$/100 μM EGTA/1 mM 2-mercaptoethanol), resuspended in buffer C containing the protease inhibitors phenylmethylsulfonyl fluoride (100 μg/ml), leupeptin (1 μg/ml), and pepstatin (1 μg/ml), and disrupted by mixing with glass beads (4°C). The extracts were centrifuged at 16,000 × *g* for 10 min (4°C). The protein content of the extracts was determined by the method of Bradford. Protein samples containing 100 μg of total protein were separated on 0.1% SDS/8% polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were probed with a monoclonal antibody (1:100 dilution) directed against the influenza hemagglutinin epitope (24). The membranes were washed with phosphate-buffered saline/Tween 20 and then incubated with alkaline phosphatase-conjugated goat anti-mouse antibody at a 1:500 dilution in blocking buffer and visualized with the 5-bromo-4-chloro-3-indolyl phosphate (BCIP) color reagent (Bio-Rad).

PDE Assays, Inhibition Studies, and Saturation Binding. Reactions were done as described (4) by using 12.5 μg of total protein per ml of reaction and various cAMP concentrations (0.01 μM–1 mM). All data points were done in triplicate. The terminated reactions were passed over an anion-exchange column (AG1-X8, Bio-Rad) which was then washed with 2 ml of 50% ethanol. The eluate was mixed with scintillation fluid (EcoLite, ICN), and radioactivity was measured by scintillation spectrometry. Recovery of [^{3}H]adenosine 5′-monophosphate was >99%, as determined with [^{14}C]AMP. Ten micromolar cAMP as substrate and various concentrations of inhibitor (0.1 μM–1 mM) were used for IC_{50} determinations. Analysis of three separate extracts from independently transformed cells gave kinetic values with a variation of <5%. Similarly, three independent determinations of IC_{50} yielded values with <10% variation. (*R*)-Rolipram was from Joseph Beavo (University of Washington, Seattle). Isobutylmethylxanthine (IBMX) and Ro20-1724 were purchased from Sigma and Biomol Research Laboratories, respectively. K_m, V_{max}, and IC_{50} values were calculated by using SIGMAPLOT (Jandel). Saturation binding experiments were done by a modification of published protocols (25). Reactions were performed at 4°C for 1 hr in 100 μl containing 50 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 50 μM 5′-AMP, and 10 mM 2-mercaptoethanol. (*R*)-[^{3}H]Rolipram (from Theodore Torphy, SmithKline Beecham) was varied from 0.10 to 25 nM. Background was <5% of signal, and the signal could be inhibited with unlabeled rolipram.

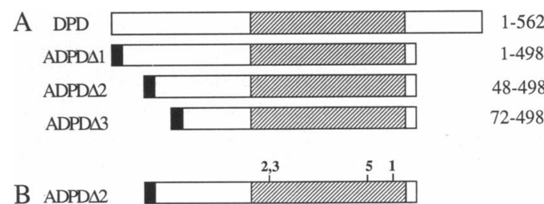

FIG. 1. (*A*) Amino-terminal deletions made in DPD sequence to define an active, rolipram-sensitive, PDE of minimum length (DPD is the original clone; ref. 4). The black box denotes the epitope tag that is part of the fusion protein; the hatched box denotes the catalytic region that is most highly conserved among PDEs. Numbers at right indicate DPD amino acid residues in the fusion protein. (*B*) Mutations in rolipram-resistant DPD mutants. Nucleotide and amino acid changes shown are as follows: mutant A1, $T^{1347} \rightarrow C$ (Val-449 to Ala); mutant A2, $G^{715} \rightarrow A$ (Asp-239 → Asn); mutant A3, $A^{716} \rightarrow C$ (Asp-239 → Ala); mutant A5, $A^{1214} \rightarrow G$ (Thr-405 → Ala). Nucleotides are numbered as in the original DPD clone (4).

RESULTS

Analysis of PDE Deletion Mutants. We created deletions of the rat clone DPD (IV_{B1}) PDE (4) to define an active type IV PDE of minimum length that could be used as a parent for isolating drug-resistant mutants. All constructs (Fig. 1*A*) include an amino-terminal epitope fusion and are expressed from an alcohol dehydrogenase 1 promoter residing on a high-copy shuttle vector. These deletion mutants were expressed in strain PP5 and were tested in a heat-shock assay. PP5 cells carry disruptions of both yeast PDE-encoding genes and, thus, have no endogenous PDE activity and are exquisitely sensitive to heat shock due to elevated levels of intracellular cAMP (19). PP5 cells expressing an active mammalian PDE are resistant to heat shock, demonstrating complementation of the PDE deficiency. All deletion mutants conferred heat-shock resistance comparable to that seen for wild type (data not shown), demonstrating that 71 amino acids from the amino terminus and 64 amino acids from the carboxyl terminus are not necessary for PDE activity. These results are consistent with other reports (26) and serve to further define the PDE catalytic domain.

Extracts from cells expressing the PDE constructs were prepared and analyzed. Table 1 shows the calculated K_m and V_{max} for constructs ADPDΔ1, ADPDΔ2, and ADPDΔ3. There is no significant alteration in the K_m of these enzymes compared with wild-type DPD sequence (4). The apparent increase in V_{max} for construct ADPDΔ3 is most easily ex-

Table 1. Kinetic properties of DPD mutant enzymes

Mutant enzyme	K_m, μM	V_{max}, nmol/min per mg
ADPDΔ1	2.40 ± 0.37	3.27 ± 0.02
ADPDΔ2	2.19 ± 0.04	5.49 ± 0.04
ADPDΔ3	2.65 ± 0.10	11.50 ± 0.18
A1	1.67 ± 0.06	4.28 ± 0.06
A2	7.27 ± 0.41	23.80 ± 0.69
A3	2.93 ± 0.09	7.81 ± 0.11
A5	17.80 ± 1.48	0.19 ± 0.01
A1/A2	2.36 ± 0.12	5.42 ± 0.12
A1/A3	1.58 ± 0.11	4.49 ± 0.12

K_m and V_{max} values for the rolipram-resistant mutants should be compared with the amino-terminal deletion mutant ADPDΔ2, from which they were generated. Each K_m and V_{max} value was calculated by using the SIGMAPLOT program from data points at six different cAMP concentrations, each in triplicate.

plained by an increase in protein levels, as visualized on an immunoblot (Fig. 2). This result may reflect an increase in protein stability resulting from the deletion. Others (26, 27) have shown that amino-terminal truncations of PDEs can lead to an increase in V_{max} that is independent of protein levels. Our mutants, which differ from those described previously, do not show this property.

Isolation of Rolipram-Resistant Mutants. To isolate PDE mutants resistant to rolipram, we first developed an *in vivo* assay to test for rolipram sensitivity. Cells were grown in YPD for 3 days and were then treated with 500 μM rolipram overnight. Long growth periods appear necessary for cells to enter the G_1 arrested (heat-shock resistant) state. These cell cultures were then heat shocked (52°C, 25 min) and plated. PP5 cells expressing a rolipram-sensitive PDE should become heat-shock sensitive when exposed to the drug because this treatment will inhibit the PDE activity. PP5 cells expressing construct ADPDΔ2 are heat-shock resistant but become sensitive after pretreatment with rolipram (Fig. 3). Accounting for culture dilution before plating, a 10^6-fold reduction in heat-shock survival occurs, reflecting sensitivity to rolipram. When this experiment was done in analogous cells that lack the *cam* mutation (28, 29), we observed a 100-fold reduction in sensitivity (data not shown). This result suggests that the *cam* mutation, originally described for its cAMP permeability, also allows uptake of quite different compounds (i.e., rolipram) by yeast cells. Although this type of broad-range permeability has been seen with other yeast mutants (30), the basis of drug uptake is not known.

The mutant ADPDΔ2 was used as a parent for random mutagenesis and isolation of rolipram-resistant PDEs. A mutant library of ADPDΔ2 was used to transform PP5 cells. These cells were grown first in selective medium (SC-Leu) and then transferred to YPD, grown for 3 days, treated with rolipram, heat-shocked, and plated. Plasmid DNA was then isolated and used to transform fresh PP5 cells. Fig. 3 shows the results of a heat-shock experiment with PP5 cells carrying the rolipram-resistant mutant A1. The mutant consistently shows a 10^4-fold increase in survival after rolipram treatment.

Four independent mutants were isolated from 5×10^5 transformants. Each mutant had a single base change (Fig. 1*B*). All mutations reside in the putative catalytic region of DPD sequence. Two mutations (A2 and A3) result in alterations of the same amino acid codon. To ascertain whether these mutations affect separate or related functions, we also constructed two double mutants, A1/A2 and A1/A3.

Kinetic Properties of Mutant Enzymes. Table 1 shows the results of biochemical analysis of extracts from yeast cells expressing mutant PDEs. The K_m values of mutants A1 and A3 show no significant change from that of the parent enzyme (ADPDΔ2). Mutant A2 shows some increase in K_m and a

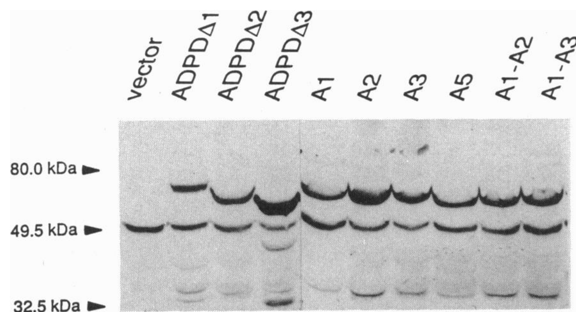

FIG. 2. Expression of DPD constructs in yeast. Extracts from PP5 cells transformed with DPD constructs or vector alone were electrophoresed on a polyacrylamide gel and transferred to nitrocellulose. The sizes of ADPDΔ1, ADPDΔ2, and ADPDΔ3 are consistent with their amino-terminal deletions, which would predict proteins of 61 kDa, 56 kDa, and 53 kDa, respectively.

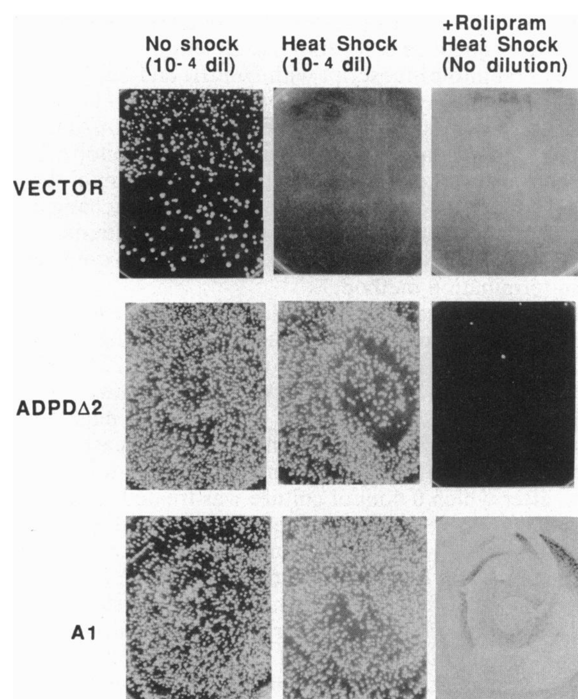

FIG. 3. Heat-shock assay. PP5 was transformed with pAD54N (vector), ADPDΔ2 (rolipram-sensitive deletion mutant), and A1 (rolipram-resistant mutant). Platings were done at a 10^4 dilution, except rolipram-pretreated cells that were plated undiluted.

large increase in V_{max}. Unlike the deletion mutant ADPDΔ3, this is not attributable to an increased amount of protein (Fig. 2). Mutant A5 displays a marked increase in K_m as well as a very large V_{max} decrease. Elevation in K_m is predicted for a mutation that directly reduces substrate binding as well as inhibitor binding. Double mutants A1/A2 and A1/A3 show K_m and V_{max} values very close to those of A1 itself.

Inhibition Studies of Mutant Enzymes. The same cell extracts used to determine kinetic parameters (above) were tested for sensitivity to rolipram. Table 2 shows IC_{50} values for parent enzyme and amino-terminal deletion mutants. The deletions do somewhat reduce inhibition of the enzyme by rolipram [note that the IC_{50} value for ADPDΔ3 construct was not adjusted to reflect its higher expression (Fig. 2)]. In particular, the largest construct was the most sensitive to the drug. All of the point mutants (Fig. 4 and Table 2) were, as expected, less sensitive to rolipram. Although mutant A1 was significantly less affected than the other mutants, even this modest reduction in rolipram sensitivity dramatically increased heat-shock survival (Fig. 3). When the double mutants were analyzed for response to rolipram, they were shown to be considerably less sensitive than either of the single mutants (Table 2). In fact, the IC_{50} for the double mutants was never reached due to the limited solubility of rolipram under the assay conditions.

To test for inhibitor-specific resistance of the mutants, two other PDE inhibitors were used. Ro20-1724 is another type IV-specific PDE inhibitor with a structure similar to rolipram. IBMX is a broad-range PDE inhibitor that is chemically distinct from rolipram and Ro20-1724 and is structurally more similar to the substrate, cAMP. Table 2 shows the IC_{50} values for the amino-terminal deletions, point mutants, and double mutants when treated with each of the three inhibitors. Because Ro20-1724 is a less potent inhibitor of type IV PDEs (16, 17), all IC_{50} values were substantially higher than those seen for rolipram. However, as shown in Table 2, the change in sensitivity of the mutants to Ro20-1724 generally parallels the changes seen for their response to rolipram. Again,

Table 2. Inhibitor effects on DPD mutant enzymes

	Rolipram		Ro20-1724		IBMX	
Mutant enzyme	IC_{50}, μM	Fold change	IC_{50}, μM	Fold change	IC_{50}, μM	Fold change
ADPDΔ1	0.4		3.9		48	
ADPDΔ2	1.6	1	10	1	64	1
ADPDΔ3	1.0		9.5		69	
A1	5.9	4	68	7	132	2
A2	633	390	>1000	>100	190	3
A3	443	270	>1000	>100	545	9
A5	533	330	761	76	>1000	>16
A1/A2	>1000	>600	>1000	>100	112	2
A1/A3	>1000	>600	>1000	>100	691	10

Values for rolipram-resistant mutants should be compared with the amino-terminal deletion mutant ADPDΔ2, from which they were generated. IC_{50} values were calculated by using the program SIGMAPLOT from data points at six different concentrations of inhibitor, each in triplicate.

mutant A1 shows a moderate effect, whereas mutants A2 and A3 are severely affected. We were unable to ascertain from this data whether the double mutants display the synergistic effect on inhibition that was seen for rolipram, because the IC_{50} values are too high to measure accurately. In contrast to their response to rolipram and Ro20-1724, mutants A1, A2, and A3 showed only a very small change in IC_{50} for the general PDE inhibitor IBMX. Most mutants, in fact, are now more sensitive to IBMX than they are to rolipram or Ro20-1724. In addition, double mutants A1/A2 and A1/A3 showed no synergistic increase in resistance to IBMX relative to the single mutants, in contrast to their increased resistance to rolipram. Finally, unlike the other mutants, A5 showed a substantial change in IC_{50} for IBMX.

Rolipram-Binding Analysis. To test whether the reduction in rolipram sensitivity was a direct reflection of reduced binding affinity, we did binding assays using (*R*)-[^{3}H]rolipram. Of the deletion mutants, all showed significant binding (data not shown). A saturation binding curve was determined for ADPDΔ1, and a K_d of 5.0 ± 0.9 nM for (*R*)-[^{3}H]rolipram was calculated from this data (the result varied somewhat with each extract and was sometimes as high as 12.4 ± 1.2 nM). This value is in general agreement with values obtained for other mammalian type IV PDE isozymes (28, 29). We did not, however, detect any of the high-affinity binding reported by others (31, 32). This result may be due to differences between PDE isozymes or because the ADPDΔ1 construct carries a carboxyl-terminal deletion and is altered at its amino terminus by the epitope tag. Each of the rolipram-resistant point mutants of DPD were then subcloned into ADPDΔ1,

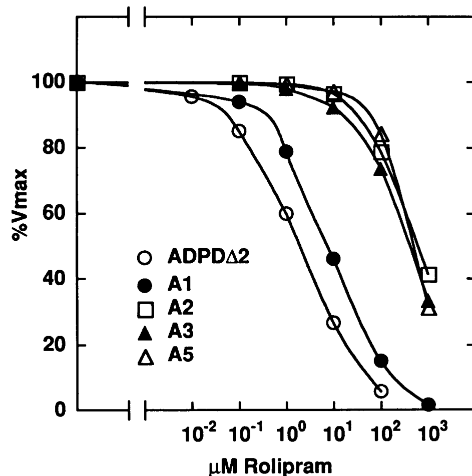

FIG. 4. Rolipram inhibition curve for drug-resistant mutants. The activity with no inhibitor present is given as 100%.

the construct that is most responsive to rolipram (Table 2). Although binding was too weak to calculate K_d value, mutant A1 showed binding that was consistently above background levels, whereas mutants A2, A3, and A5 showed very little binding (Table 3). This result indicates that resistance to rolipram is, at least in part, mediated through a reduction in binding affinity.

DISCUSSION

We describe here the use of an *in vivo* yeast model system to study the interactions between a mammalian PDE and competitive inhibitors of pharmacological importance. Analysis of deletion mutants indicates that most of the residues downstream of the catalytic region do not affect kinetic properties and are not required for rolipram responsiveness. At the amino terminus, deletions up to 71 amino acids had very little effect on substrate K_m and V_{max} (except for ADPDΔ2, which showed a higher V_{max} corresponding to higher enzyme levels). The amino-terminal deletions reported here do have a small effect on the ability of the enzyme to interact with rolipram, as judged by IC_{50} measurements, suggesting the presence of minor binding or structural determinants in this region.

The protocol used to select drug-resistant PDEs allowed us to isolate enzymes that still react with substrate but are now resistant to inhibitory drugs. Fig. 5 shows a model for rolipram binding to a PDE. Mutations at position A would be expected to affect both substrate and inhibitor binding, whereas position B mutations might only block inhibitor binding. Mutant A5 appears to fall into category A, as judged by its diminished substrate kinetics and its resistance to IBMX. Although they each have specific properties, mutants A1, A2, and A3 behave as category B mutants because their substrate kinetics are not reduced. Interestingly, mutant A2 actually shows a 4-fold increase in V_{max} that is not the result of increased protein levels (Fig. 2). This result could reflect an alteration in the enzyme interaction with its normal substrate, product, or transition state. The double mutants A1/A2 and A1/A3 show kinetic properties that are intermediate for those seen with the parent mutants but show a

Table 3. (*R*)-[^{3}H]Rolipram binding to DPD mutant enzymes

Mutant	Binding, fmol/mg
Vector	18.3 ± 1.2
ADPD	535.6 ± 19.2
A1	46.9 ± 6.0
A2	26.5 ± 3.3
A3	22.1 ± 2.1
A5	28.0 ± 5.6

Mutants are in the ADPDΔ1 background. (*R*)-[^{3}H]Rolipram was used at 20 nM. Each value represents the mean of five experiments.

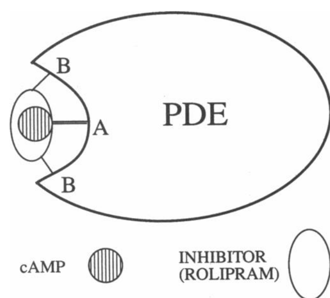

FIG. 5. Model for the possible location of mutations conferring rolipram resistance.

synergistic effect on IC_{50} for rolipram. This result is consistent with a model where two separate and cooperative interactions have been disrupted.

The effectiveness of Ro20-1724 on the deletion and point mutants paralleled that seen for rolipram. This similarity was not surprising, given their relatedness of structure. Distinct properties of the point mutants were revealed by using IBMX. A5, the mutant most affected in substrate binding, is also most affected in response to IBMX, the inhibitor that most resembles the substrate. Mutants A2 and A3 are specifically disrupted in their interactions with rolipram and Ro20-1724 but are still subject to IBMX inhibition, implying that the mutations block an interaction with a feature specific to the former compounds. It should be noted that the mutated residues are neither specific to type IV PDEs nor are they completely conserved among all known PDE isoforms. Because no direct structural data exists for any PDE, this analysis does not allow us to ascertain whether the mutated residues are actual sites of contact with the inhibitor or if the mutations result in structural changes that interfere with contact at other residues.

One striking aspect of the mutant analysis is the difference in magnitude of the *in vitro* IC_{50} changes compared with the *in vivo* heat-shock survival assay results. Mutant A1 is notable in this respect. Its IC_{50} for rolipram changed by only 4-fold, whereas survival changed by 10^4-fold. This result resembles the behavior of 3′-azido-3′-deoxythymidine (commonly referred to as AZT)-resistant human immunodeficiency virus reverse transcriptase, which shows only very slight biochemical changes from the wild-type enzyme *in vitro* (33, 34). For the PDE type IV enzyme expressed in yeast, a difference between biological and pharmacological potency presumably reflects the fact that survival requires only a relatively small amount of PDE activity; this is demonstrated by the heat-shock survival of cells carrying mutant A5, which produces only ≈5% of the activity of the parent enzyme. These results suggest that biological assays in microorganisms may be a very fruitful approach for the study of inhibition of mammalian enzymes.

The system described here should be generalizable to the study of other mammalian enzyme systems that can be expressed in yeast. This analysis can be extended to the isolation of drug-resistant mutants of other mammalian proteins or simply used for the rapid screening of new drugs with inhibitory potential. The system could involve either the replacement of a yeast gene with its mammalian homologue, as described here, or simply the overexpression of a mammalian gene product that can interface with an existing yeast pathway to elicit a selectable phenotype. Mammalian transcription factors, which have been demonstrated to work in yeast (35, 36), are excellent subjects for such analysis, which could lead to other avenues of drug therapy (37).

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