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Molecular cloning of rat homologues of the *Drosophila melanogaster* dunce cAMP phosphodiesterase: Evidence for a family of genes

(cAMP/spermatogenesis/germ cells/Sertoli cell)

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ABSTRACT **To study the structure and function of cyclic nucleotide phosphodiesterases (PDEs) involved in mammalian gametogenesis, a rat testis cDNA library was screened at low stringency with a cDNA clone coding for the *Drosophila melanogaster* dunce-encoded PDE as a probe. This screening resulted in the isolation of two groups of cDNA clones, differing in their nucleotide sequences (ratPDE1 and ratPDE2). In the rat testis, RNA transcripts corresponding to both groups of clones were expressed predominantly in germ cells. Additional screenings of a Sertoli cell cDNA library with a ratPDE2 clone as a probe led to the isolation of two more groups of clones (ratPDE3 and ratPDE4). Unlike ratPDE1 and ratPDE2, these clones hybridized to transcripts present predominantly in the Sertoli cell. In the middle of the coding region, all four groups of clones were homologous to each other. The deduced amino acid sequences of part of this region were also homologous to the *D. melanogaster* dunce PDE and to PDEs from bovine and yeast. These data indicate that a family of genes homologous to the *D. melanogaster* dunce-encoded PDE is present in the rat and that these genes are differentially expressed in somatic and germ cells of the seminiferous tubule. These findings provide a molecular basis for the observed heterogeneity of cAMP PDEs.**

Intracellular cAMP is regulated at the level of synthesis by adenylate cyclase and at the level of degradation by cyclic nucleotide phosphodiesterases (PDEs). These latter enzymes form a large family and differ in affinity for cAMP and cGMP, in subcellular localization, and in regulation by hormones and second messengers (1).† Most cells contain more than one PDE, and some cell types have different forms than others (1). Although several studies with PDE inhibitors (3) or mutant PDEs (4) provide a clue on the involvement of specific PDE forms in various physiological processes, the molecular basis for this heterogeneity is largely unknown.

Several observations suggest that regulation of intracellular cAMP levels in gonadal cells plays a prominent role in the gametogenic process and that PDEs are involved in this regulation. In *Drosophila melanogaster,* mutations in a dunce (*dnc*) locus, coding for a cAMP PDE, produce female flies that are sterile (5). A decrease in intracellular cAMP levels, which may be caused by the activation of a PDE, is associated with meiotic resumption of amphibian and mammalian oocytes (6). In the rat, a cAMP PDE is regulated during the ovarian cycle and during testicular development (7, 8). This PDE is activated by the follicle-stimulating hormone in the granulosa cell and the Sertoli cell (9, 10). It has also been shown that spermatozoan motility is dependent on intracellular cAMP levels since it is augmented by treatment with PDE inhibitors such as isobutylmethylxanthine (11).

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The heterogeneity, instability, and relative low abundance of some of the PDE forms have been major obstacles in purifying and characterizing these enzymes. Here we report an alternative approach to study the structure, the role, and the regulation of PDEs involved in mammalian gametogenesis. A rat testis cDNA library was screened with a *D. melanogaster* dunce-encoded PDE cDNA clone (4), and one of the isolated clones was used to rescreen a Sertoli cell cDNA library. These screenings resulted in the isolation of four groups of cDNA clones homologous to the *D. melanogaster* dunce PDE and corresponding to four different genes.‡

MATERIALS AND METHODS

Screening of a Rat Testis and a Rat Sertoli Cell cDNA Library. A testis cDNA library in λgt11, constructed with poly(A)$^+$ RNA from 28-day-old Sprague–Dawley rats (12), was screened with a random-primed ^{32}P-labeled (13) *D. melanogaster* dunce cDNA clone (4) (provided by R. L. Davis, Baylor College of Medicine, Houston). Hybridization conditions were as described (14), except that 30% (vol/vol) formamide was used at 37°C and the final stringent wash was at room temperature. One positive plaque was purified, the recombinant DNA was isolated (15), and the *Eco*RI insert was subcloned into plasmid pGEM-3Z (Promega) for propagation. A 1-kilobase (kb) fragment of this insert, which contained only coding sequences, was labeled and used as a probe to rescreen the library at higher stringency. One of the isolated rat PDE clones (ratPDE2.1) was used as a probe for the screening of a Sertoli cell cDNA library. This library was constructed with poly(A)$^+$ RNA (16–18) from cultured Sertoli cells from 15-day-old Sprague–Dawley rats that, on the fourth day of culture, had been treated with 0.5 mM N^6, $O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate. Hybridizations were done in a solution containing 30% formamide at 37°C, and washes were performed at 50°C.

DNA Sequencing. Restriction fragments and random fragments (19) of the *Eco*RI inserts of the positive clones were subcloned into pGEM-3Z or into M13. The nucleotide sequences were determined using the dideoxy chain-termination method of Sanger *et al.* (20) with ^{35}S-labeled deoxyadenosine 5′-[α-thio]triphosphate. Compressions were resolved by using 7-deaza-2′-deoxyguanosine triphosphate in place of dGTP (21). The sequences were analyzed by using routines available with the MicroGenie (Beckman) software package.

Cell Isolation. Sertoli cell-enriched cultures, peritubular cell cultures, and total germ cell suspensions were prepared as

Abbreviation: PDE, cyclic nucleotide phosphodiesterase.

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†Based on these criteria, PDEs have been classified into four types: type I, calmodulin-sensitive PDEs; type II, cGMP-sensitive PDEs; type III, rhodopsin-sensitive cGMP PDEs; type IV, cAMP PDEs, also named low K_m cAMP PDEs or PDE III (2).

‡The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M25347 (ratPDE1), M25348 (ratPDE2), M25349 (ratPDE3), and M25350 (ratPDE4)].

described (22). Sertoli cell-enriched testes were prepared by *in utero* irradiation on the 19th day of pregnancy according to a previously reported method (23). The effect of irradiation was monitored by histological examination of testicular sections.

RNA Isolation and Analysis. Total RNA (16) or poly(A)$^+$ RNA (17) was prepared from tissue homogenates or from cell cultures, glyoxylated, and fractionated by electrophoresis through a 1% agarose gel. The RNA was transferred onto nylon membranes (ICN) (24) and analyzed by hybridization with random-primed ^{32}P-labeled (13) cDNAs.

RESULTS

cDNA Cloning and Sequencing of Rat Testis Homologues of the *D. melanogaster* Dunce-Encoded PDE. A rat testis cDNA library in λgt11 was screened at low stringency with a *D. melanogaster* dunce-encoded PDE cDNA as a probe. The initial screening resulted in the isolation of one positive clone (ratPDE1.1) out of ≈2 × 10^5 recombinants. A 1-kb fragment of this clone was used to rescreen the library at higher stringency. Two of the five positive clones from this second screening (ratPDE1.2 and ratPDE1.3) produced a strong signal, whereas the three other were only weakly positive and were tentatively included in a second group of clones (ratPDE2.1, ratPDE2.2, and ratPDE2.3). These latter clones were strongly positive when hybridized with a cDNA similar to the *Drosophila* dunce cDNA isolated from a rat brain cDNA library (provided by R. L. Davis). Three clones from these two screenings (ratPDE1.1, ratPDE1.2, and ratPDE2.1) were sequenced. Comparison of the nucleotide sequences demonstrated that ratPDE1.1 and ratPDE1.2 had identical

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                15          30          45          60          75          90          105         120
ratPDE1  CCCATGGCCCAGATCACTGGGCTGCGGAAGTCTTGCCACACC  AGCCTTCCTACAGCTGCCATCCCGCGCTTTGGGGTCCAGACAGACCAGGAGGAGCAGCTGGCTAAGGAGCTTGAA
ratPDE2  ......T.T........A......AAA...CTGGTA.....TGGA...T.G  .ACA.CAA.G....A..G.....A...A.......T..A.....C.TCT.A..AC.A..A..G..G
ratPDE3  ..G...T.A.......G....G.CAA.....TGATG....G.TCC.....GA.C.ATT.CTG...T..AA.A........TA.A.....G.....A..TGTC.....C.....A..A...
ratPDE4  .T....A.......A.G...AG..AA...ACTGATG....G.TCA.....GAAC.ACA.AAG....T.A........A...A.C..G..AA.T.....T..T..A..C........G...

                135         150         165         180         195         210         225         240
ratPDE1  GACACCAACAAATGGGGACTCGATGTGTTTAAAGTGGCTGAGCTGAGTGGGAACCGGCCCCTCACTGCTGTTATCTTCAGCGTTCTCCAGGAACGGGACCTGCTCAAGACGTTCCAGATC
ratPDE2  A..TTG.G.........C..GA.CA.C...TGT...T.G...TACGC...AGG...CT.A....GCTG.A.C..G.ATACGA.AT.......G........A..G....AA.....C...
ratPDE3  ...GTG.....G.....C...C.C..T..CCGAA.A..G......TC...C........T..G....T.A.C..GCA..C.A..T.T.........A..TT..T.A..A.....TA.A...
ratPDE4  ...CTG...........C..TA.CA.C..C..C.......G.TACTCCCAT..T...........ATGCA.C..G.A.GC.A..T.........A.A.....T..A.........TA.A...

                255         270         285         300         315         330         345         360
ratPDE1  CCGGCGGACACGCTGCTGAGGTACCTGCTGACCCTCGAAGGGCACTACCACTCCAACGTGGCGTACCACAACAGCATACATGCTGCCGACGTGGTGCAATCCGCGCACGTGTTGCTGGGC
ratPDE2  ..T.T......CA..A...T....A..........G..G.AC........TG..G.......C............C.G..C..A..G..T...C....G...A.A......C......C.
ratPDE3  ..A.T......TT..A.T.C...T..TA....T..A....AC..T.....TG.TG.......C..T.......A...C........A..T..C..C..G..AA.T..T...C....CTCT
ratPDE4  T.CT.C.....CT.CG.A.CC...A..A....TT.A....AC..T.....T..TG.T........T.........C.G..C.....T.......CC..G..AA.......TC.C..CTCT

                375         390         405         420         435         450         465         480
ratPDE1  ACCCCAGCCCTGGAGGCTGTGTTCACGGACCTAGAAGTCTTGGCCGCCATCTTTGCGTGCGCTATACACGATGTAGACCACCCAGGTGTCTCCAACCAGTTTCTCATCAACACCAACTCG
ratPDE2  ..G..C..A.....C...........A.....G..GA.TC.T..T...C....C..TGCT..C..C........G........T.CGC.............C..A...........T...
ratPDE3  ..A..C..TT..........T.....T...T.G..GA.TC.C..G.....T.....CA.T..A.....T.....G..T..T..T.....G..A..T..A.....G.....T..A......
ratPDE4  ..G.....A.....T.....C.....A.....G...A..C....T.....T.....AGCT..C..C..T.....T..T..T..T..A........T.............T..A..T..C

                495         510         525         540         555         570         585         600
ratPDE1  GAGCTGGCACTGATGTACAATGACTCATCGGTGCTAGAGAACCATCACCTCGCGGTAGGCTTCAAGCTCCTGCAGGGGGAAAACTGCGACATCTTCCAGAACCTCAGCACCAAGCAGAAA
ratPDE2  .........T..........C..TGAG..T.....T........C.....G..T..G..A........G.....A.AA..G.............................AGCGC...CGG
ratPDE3  ..A..T..CT..........C.....C..C..CT.......T.....TT.G..T..G.....T...T.G..C....AA........T.....T........T..G.C..AA.....A.GA
ratPDE4  ..A..T..TT.......T......GA...T.....G..A..............T..G..A...........T..A.A....C.T...........T.....T..T.C..AG.....ACGC

                615         630         645         660         675         690         705         720
ratPDE1  CTGAGTCTGCGCAGGATGGTCATAGACATGGTACTGGCCACAGACATGTCCAAGCACATGAGCCTCCTGGCTGACCTCAAGACCATGGTGGAGACCAAGAAAGTGACTAGCCTTGGCGTC
ratPDE2  .A...C.......A.........C........G.....................A.......C..............G.....T...........G...........C..CTCC..A..T
ratPDE3  .AATC.T.AA.G.AA....C...T.....T.....A..G...........A..........AT..G........T..G..A..A.....T..A..G.....G.....G...TC.......
ratPDE4  .A..CA..CA.G.AA.....G..T........GT.A..A..T..T.................................T..A..G.....A..A.....A..G.....G...TCC..T..T

                735         750         765         780         795         810         825         840
ratPDE1  CTGCTCTTGGACAACTACTCTGACCGCATCCAGGTCCTCCAGAGCCTGGTGCACTGCGCCGACCTCAGCAACCCTGCCAAGCCACTACCCCTCTACCGCCAGTGGACGGAGCGCATCATG
ratPDE2  ..CT.GC.....................T.............AG..A.A..........T..A...........T..CA.......C..GGAG..G.....A........C..C.........
ratPDE3  ..C...C.T..T.....T......A.G...............ATA..........T..A.....G........CA.A........C.AG.....................C..G..A...
ratPDE4  ..C...C..........TA.......G..A.....T..T.GC.A.A....A..T..T..A.....G.........A.....T.CT.GGAGT.G..T..G..A.....T..T.........

                855         870         885         900         915         930         945         960
ratPDE1  GCTGAGTTCTTCCAGCAGGGTGACCGGGAACGTGAGTCGGGCTTGGACATCAGCCCCATGTGCGACAAGCACACAGCCTCGGTGGAGAAATCCCAGGTGGGATTCATTGACTACATCGCT
ratPDE2  ...................CGC....A.....G...CGT..AA....G.............................T.........G..T.........C.....C.........T.T.
ratPDE3  .AG..........GT.....G........G......CGT...A....G..A..T........T..........AC.....T.....A.....A........C.....A.........T.T.
ratPDE4  .AG.....T.....A.....A...AAA.....G...AG...AA....G..T.....A.....T..T..A........T..T.....A..G........T..T.............T.TC

                975         990         1005        1020        1035        1050        1065        1080
ratPDE1  CACCCATTGTGGGAAACTTGGGCCGACCTGGTGCACCCCGATGCCCAGGAGCTGCTGGATACCTTGGAAGACAACAGAGAGTGGTATCAGAGTAGGGTTCCCTGCAACCCTCCACACGCC
ratPDE2  ..T...........G..A.....A..T..C..C.....G........A..CA.C.....C..GC...........C.G..C.....C..C...GCCA...GGCAG.GT...T.C.CAC..
ratPDE3  ..T...C.C.....G..A.....A.....C..A..T.....C........CA.TT....C..T.....G.....TC.T........C.....C.CAA.C...CAG.G...CT.C.CT..A
ratPDE4  ..T...........G..C.....A........T..G..T.....T..A..CA.TT....C..AC.A.....T.....GA.C.....C.......T.A.....CAG.G...CT.T.CAC.A
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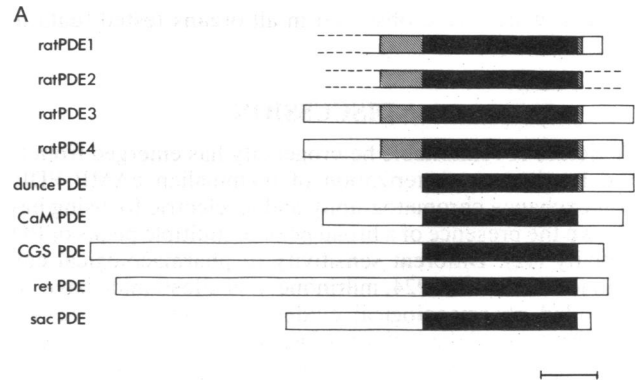
FIG. 1. Comparison of the nucleotide sequences from four groups of cDNA clones isolated from testicular cell libraries. The nucleotide sequences of ratPDE1, ratPDE2, ratPDE3, and ratPDE4 are aligned. A core of 1080 bases of the coding region was found to be homologous in all four groups of clones and is reported herein. Dots in the sequences represent identity of a nucleotide with that of ratPDE1. One gap of three bases has been introduced in ratPDE1 and in ratPDE2 to optimize the alignment. Horizontal arrows delimit the region from which the deduced amino acid sequence is compared in Fig. 2. Nucleotides are numbered on the top starting from the first nucleotide shown.

sequences in the overlapping region and that ratPDE2.1 was homologous but not identical (Fig. 1). Under high-stringency conditions, none of these clones detected transcripts in the Sertoli cell of the rat testis (vide infra), which, however, is known to contain at least two PDE forms (10). Therefore, the ratPDE2.1 clone was used to screen a Sertoli cell cDNA library at low stringency. Five more cDNA clones were plaque purified. One of these five clones (ratPDE3.1) was labeled and used in a cross-hybridization experiment at high stringency. This demonstrated that ratPDE3.1, ratPDE3.2, and ratPDE3.3 shared identical sequences, while the two other clones probably represented a fourth group (ratPDE4.1 and ratPDE4.2). Sequencing of these clones and comparison with each other confirmed that we had indeed isolated four different groups of clones (ratPDE1, ratPDE2, ratPDE3, and ratPDE4) that in a region of 1080 bases had similar but not identical nucleotide sequences (Fig. 1). Outside this region, some of the sequences were considerably different (data not shown).

Comparison of the Deduced Amino Acid Sequences of the Four Groups of Clones with One Another and with Sequences of PDEs of Various Species. Comparison of the deduced amino acid sequences of these four groups of clones showed a high degree of sequence similarity (Fig. 2 *A* and *B*). The sequence similarity was greater than that at the nucleotide level. This was explained by the observation that many nucleotide differences in the various clones were located in the third position of the codons or in positions that did not result in a change in the amino acid residue. Homology was also evident when the deduced amino acid sequences of the four rat clones were compared with sequences of PDEs from various species (Fig. 2 *A* and *B*). A region of about 270 residues is conserved in all the published PDE sequences (4, 25–27) [except for a *Dictyostelium* PDE (29)], while, on the basis of available sequences, similarities become negligible toward the NH_2 and the COOH termini. In the middle of this conserved region, the sequence Glu-Leu-Ala-Leu-Met-Tyr-Asn is present (Fig. 2*B*) in all the rat clones. This sequence is also found in the cAMP-binding domain of the RII_a regulatory subunit of the cAMP-dependent protein kinase (30, 31). Similar findings have been reported for the *Drosophila* dunce clone (4).

Distribution of Rat PDE Transcripts in the Various Cell Types of the Seminiferous Tubule. Northern blot analysis of RNA from Sertoli cell-enriched cultures, peritubular cell-enriched cultures, and germ cell suspensions with ratPDE1 and ratPDE2 as probes showed the presence of transcripts mainly in germ cells (Fig. 3). Faint hybridization signals with



B

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ratPDE1   QIPADTLLRYLLTLEGHYHSN-VAYHNSIHAADVVQSAHVLLGTPALEAVFTDLEVLAAIFACAIHDVDHPGVSNQFLINTNSELALMYNDS-SV
ratPDE2   H..V..MMM.M....D...AD-......L.....L..T....A....D.......I...L..A.........AL.....................E-..
ratPDE3   K..V...IT..M...D...AD-.....N.........T....S.............I......S.....................................-..
ratPDE4   K.SS..FVT.MM...D....D-......L.....A..T....S....D.......I......A.....................................E-..
dunce-PDE M..PK.F.NFMS...D..VKD-NPF...L.....T..TN...N.....G...P...GG.L..AC.........LT....V.SS.........E-..
CaM-PDE   K..VSC.IAFAEA..VG.XKYKNP...L......T.TV.YIMLGTGIMHWL.E..I..MV..A....YE.T.TT.N.H.QXR.DV.IL...R-..
cGS-PDE   K.DCP..A.FC.MVKKG.-RD-IP...WM..FS.SHFCYL.YKNLE.TNYLE.M.IF.LFIS.MC...L..R.TN.S.QVASK.V...AL.SSEG..
ret-PDE   H..QEA.V.FMYS.SKG.-R-RIT...WR.GFN.G.TMFS..V.GK.KRY.....A..MVT.AFC...I..R.TN.LYQMKSQNP..KLHG-.-.I
sac-PDE   L.ADNK..LL.F...SS..Q-VNKF..FR..I..M.ATWR.C-.YL.KDNPVQT--.LLCM.AIG...G...TN..L.C.CE..V.QNFKNV-.I

ratPDE1   LENHHLAVGFKLLQGENCDIFQNLSTKQKLSLRRMVIDMVLATDMSKHMSLLADLKTMVETKKVTSLGV----------LLLDNYSDRIQVLQ
ratPDE2   ..............E.........KR.RQ...K.............T.............S..----------.............R
ratPDE3   ..............E.........TK..RQ...K.A..I.........N.............S..----------.....................
ratPDE4   ..............E.H.......TK..RQT..K.............................S..----------......T......R
dunce-PDE ........A.....NQG....C.MQK..RQT..K....I..S.....................AGS..----------......T......E
CaM-PDE   .....VSAAYR.M.E.EMNVLI...KDDWRD..WL..E...S....G.FQQIKNIRNSLQ----QPE.L----------------.KAKTMS
cGS-PDE   M.R..F.QAIAI.NTHG.N..DHF.R.DYQRMLDLMR.II....LAH.LRIFK..QK.A.---------.G---------YDRT.KQHHSLL.C
ret-PDE   ..R...EF.KT..RD.SLN.....NRR.MEHAIH.MDIAII...LAL---YCKK-R..FQ-.I.DQSKTYETQQEWTQYMM..QTRKE.VMAM
sac-PDE   ...F.REL.QQ..SEH-WPLKLSI.K.KF----DFISEAI.....AL.SQYEDR----------.MHE.PMKQ.-T.I

ratPDE1   SL-VHCADLSNPAKPLPLYRQWTERIMAEFFQQGDRERESGLDI-SPMCDKHTAS-VEKSQV-GFIDYIAHPLWETWADLVHPDAQELLDTLEDN
ratPDE2   NM-.........T...E......D.........AH....R.ME.-.........-......-......V.............DI.......
ratPDE3   NM-.........T...Q......D...E...R.......R.ME.-.......N..-......-......V.............DI.......
ratPDE4   NM-.........T.S.E......D...E.......K...R.ME.-.........-......-......V...........Q....DI.......
dunce-PDE N.-.........T......KR.VALL.E...L...K.....M..-.....R.N.T-I.....-......V........S.....DI.....E.
CaM-PDE   LI-L.A..I.H...SWXKLHR..MAL.E...L...K.A.L..PF-..L..RKSTM-.AQ..I-....F.VE.---.FSL.TDSTEKIIIPLI.ED
cGS-PDE   L.-MTSC...DQT.----------K...S...L.KAM.NRP-ME.M.REK.Y-IPEL.I-S.MEH..M.---IYKL.QDLFPKAA-ELY.RV
ret-PDE   MM--TAC...AIT..WEVQSKVALLVA...WE...L..TVLQQNPI..M.RNK.DELP.L..-....FVCTFVYKEFSRF-.EEITPM..GITN.
sac-PDE   ..IIKA..I..VTRT.SISAR.AYL.TL..NDCALL.TFHKAHR--.EQ.CFGD.--Y.-N.DSPKEDLESIQNILVNVTD-..DI-IK.HPHIP
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FIG. 2. Homology between the deduced amino acid sequences of the four groups of rat cDNA clones with the sequences of PDEs from various species. (*A*) Schematic representation of the coding regions of the four groups of rat PDE clones (ratPDE1, ratPDE2, ratPDE3, and ratPDE4), the *Drosophila* dunce-encoded cAMP PDE (dunce PDE) (4), a calmodulin-sensitive PDE from bovine brain (CaM PDE) (25), a cGMP-stimulated PDE from bovine heart (CGS PDE) (25), the α subunit of a rhodopsin-sensitive cGMP PDE from bovine retina (ret PDE) (26), and a cAMP PDE from *Saccharomyces cerevisiae* (sac PDE) (27). A region of homology of about 270 amino acid residues is represented by black bars. Hatched bars indicate additional regions that are homologous (nucleotide sequences shown in Fig. 1). Other parts of the proteins are represented by open bars, and unknown regions are indicated by broken lines. The bar represents 100 amino acid residues. (*B*) Comparison of the deduced amino acid sequences of the four groups of rat PDE clones with several PDEs from various species in this region of about 270 amino acid residues. A dot in the sequence indicates identity of the residue with that of ratPDE1. Gaps (−) are introduced for alignment. Residues that are conserved (28) in all compared sequences are marked with an asterisk (groupings were as follows: A, S, and T; N and Q; D and E; R, K, and H; I, L, M, and V; F, W, and Y; G; P; and C). Two asterisks on the top of a residue indicate the identity of that residue in all the compared sequences. Amino acids are identified by the single-letter code.

ratPDE2 were also detected in peritubular cells (Fig. 3). The ratPDE3 clones hybridized to transcripts from Sertoli cells that had been treated with follicle-stimulating hormone, as described elsewhere (unpublished data). Transcripts corresponding to ratPDE4 were readily detectable in the Sertoli cell (Fig. 3), while faint hybridization signals were also present in peritubular and germ cell RNA. It is not clear if this latter finding is due to the presence of low levels of these transcripts in these cells, to Sertoli cell contamination in the peritubular cell cultures and in germ cell suspensions, or to cross-hybridization of ratPDE4 to other rat PDE transcripts. That germ cells are the major site for expression of ratPDE1 and ratPDE2 in the seminiferous tubule was confirmed by the absence of hybridization signals in testes after *in utero* irradiation (Fig. 4). This treatment depletes the testis of meiotic and postmeiotic germ cells and enriches it in Sertoli cells (23). Hybridization to ratPDE3 and ratPDE4 was retained or increased in the testes from irradiated rats, confirming their predominant presence in somatic cells (Fig. 4). The observation that the levels of transcripts of ratPDE1 and ratPDE2 are low in testes from 16-day-old immature rats and increase during testicular maturation (data not shown) is in agreement with these findings.

Organ Distribution of RNA Transcripts Corresponding to the Various Groups of cDNA Clones. To assess if transcripts hybridizing to the rat PDE cDNA clones are present only in the testis or if they are also expressed in other organs, poly(A)$^+$ RNA from brain, heart, liver, kidney, and testis was hybridized with members of the four groups of cDNA clones. With ratPDE1 as a probe, major hybridization signals were found in the testis (4.2 kb, 3.2 kb, and 2.2 kb) and in the kidney (4.2 kb and 3.6 kb) (Fig. 5). A ratPDE2 probe showed transcripts in all examined organs except in liver (4.5 kb and 4.0 kb in the brain; 4.5 kb in the heart and the kidney; and 4.5 kb, 4.0 kb, 3.5 kb, and 3.1 kb in the testis) (Fig. 5). The hybridization signal in the kidney was weak. All tested organs showed transcripts corresponding to ratPDE4 (4.6 kb, 4.2 kb, and 3.9 kb in the brain; 4.5 kb and 4.0 kb in the heart; and 4.0 kb in the liver, the kidney, and the testis) (Fig. 5). With this probe, faint signals were observed between 7.4 and 5.8 kb.

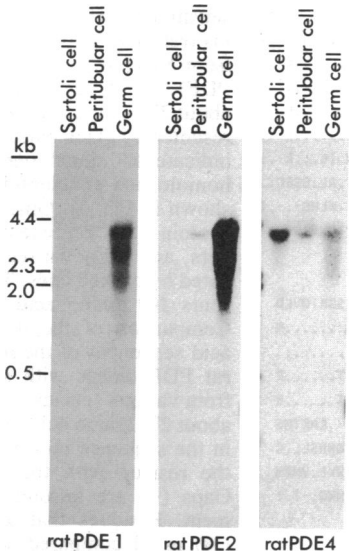

FIG. 3. Distribution of rat PDE transcripts in the various cell types of the seminiferous tubule. A Northern blot with 20 μg of total RNA from Sertoli cell-enriched cultures, peritubular cell-enriched cultures, and germ cell suspensions was hybridized (24) with a ^{32}P-labeled rat PDE clone. After autoradiography, the probe was removed, and the blot was rehybridized with the other rat PDE clones. Size markers were ^{32}P-labeled *Hin*dIII-digested λ DNA and *Hae* III-digested ϕX174 DNA fragments.

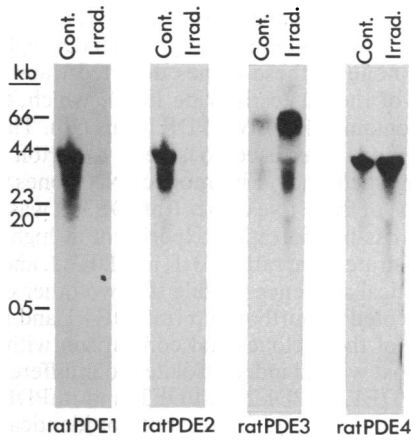

FIG. 4. RNA analysis of control testis and of testis from irradiated rats with the various rat PDE clones. A Northern blot with 20 μg of total RNA from testes from 29-day-old control (cont.) rats and from 29-day-old rats that had been irradiated *in utero* (irrad.) was hybridized with ^{32}P-labeled rat PDE cDNA clones as described in the legend of Fig. 3 and autoradiographed.

This is the result of cross-hybridization with ratPDE3 transcripts, which were observed in all organs tested (data not shown).

DISCUSSION

A picture of remarkable heterogeneity has emerged from the biochemical characterization of mammalian cAMP PDEs. Ion-exchange chromatography and isoelectric focusing have shown the presence of a broad peak or multiple peaks of PDE activity (32). Different sensitivity to pharmacological compounds like Ro 20-1724, milrinone, and cilostamide have also provided pharmacological evidence for this heterogeneity (3). Because of the lack of information on protein structure and sequence of any of these forms, little is known about the cause of this diversity. Here we report the molecular cloning of four groups of cDNA clones of rat testis origin with considerable sequence similarity to the *D. melanogaster* dunce cAMP PDE (4). This finding together with the notion that transcripts are differentially expressed in the various cell types of the seminiferous tubule and in several other organs in the rat indicates the presence of at least four PDE genes.

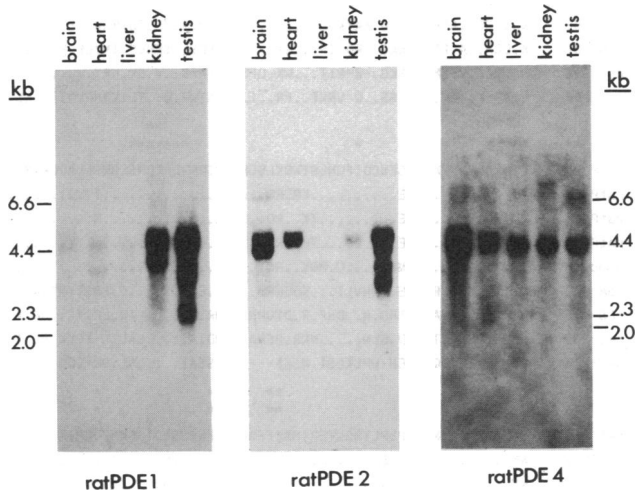

FIG. 5. Detection of rat PDE transcripts in various organs of the rat. A Northern blot with 5 μg of poly(A)$^+$ RNA from brain, heart, liver, and kidney from 90-day-old rats and testes from 29-day-old rats was hybridized with a ^{32}P-labeled rat PDE cDNA clone as described in the legend of Fig. 3 and autoradiographed.

This provides a molecular basis for the observed cAMP PDE heterogeneity. Since mutations in the *Drosophila* dunce cAMP PDE result in sterility of female flies (5), the reported homology of the rat PDE genes with the *Drosophila* gene points to an important role of the products of these genes in the gametogenic process.

Upon comparison of the nucleotide sequences, there was not a region longer than 32 nucleotides in which two of the four groups of cDNA clones had identical nucleotide sequences. This finding indicates that these groups of clones represent transcripts from different genes, rather than being derived from a single gene by alternative processing. Considering the complex gene arrangements found in the dunce locus of *Drosophila* (33), it will be interesting to further investigate whether these genes are, in the genome, in close spatial relationship. Another point that needs to be clarified is whether all four genes are encoding functional enzymes. It should be pointed out that all these genes are expressed at the level of RNA transcripts. In addition, most nucleotide differences between the various clones are located in positions that cause conservative or, in many cases, no amino acid residue changes. If one of the clones were not encoding a functional enzyme, we would expect the nucleotide differences to be more randomly distributed. The open reading frames found in the rat clones terminate at positions similar to those found in other homologous PDEs from various species. Finally, ratPDE1, ratPDE3, and ratPDE4 cDNAs used for transformation of bacteria resulted in an increased PDE activity of the bacterial extracts, indicating that these cDNAs are derived from transcripts encoding functional enzymes (unpublished results).

In addition to the data on bacterial expression, several other lines of evidence support the idea that these four groups of cDNA clones encode PDEs. In a region of about 270 amino acid residues, the deduced amino acid sequences of the four groups of clones are homologous to PDEs from various species ranging from yeast to bovine. In this region, 22 residues, 5 of which are histidines, are identical in all nine sequences compared. This suggests that these residues are involved in the catalytic activity of the enzyme or play a role in its folding. The homology with a cDNA clone coding for the *D. melanogaster* dunce PDE, the clone that we used as a probe for the initial screening, is striking. This PDE has a high affinity for cAMP and can therefore be classified as a low K_m cAMP PDE (4). The rat clones and the *Drosophila* clone (4) have a region of perfect sequence similarity with the cAMP binding domain of the regulatory subunit of the cAMP-dependent protein kinase, suggesting that this region of the PDEs is involved in the binding of cyclic nucleotides. Furthermore, we have direct evidence that at least one of the rat clones (ratPDE3) encodes a cAMP PDE (unpublished results). In view of the observed homology, it is likely that the other three rat PDE genes also encode cAMP PDEs.

Our localization studies of rat PDE transcripts in the different cell types of the seminiferous tubule indicate that ratPDE1 and ratPDE2 are predominantly expressed in the germ cells, whereas the other two genes (ratPDE3 and ratPDE4) hybridize mainly to the RNA from Sertoli cells. These data are in accordance with the biochemical evidence that in the testis germ cells possess PDEs different from those present in testicular somatic cells (8). They indicate that the expression of this family of genes is different in different testicular cell types. Since more than one PDE gene is expressed in the other organs tested, it is possible that different cells within any organ express different PDE genes. The physiological significance of this differential expression is at present unknown. The COOH and the NH_2 termini might modulate or modify the catalytic properties of these PDEs and/or specify their subcellular distribution. In these two regions, the four groups of PDE clones display little or no sequence similarity to each other, whereas the core region is highly conserved. The reported presence of multiple transcripts might add a further level of complexity to these regions.

The four isolated groups of cDNA clones provide powerful tools to further study the regulation of their corresponding genes and encoded proteins and might be useful in the development of more specific drugs that act as PDE inhibitors. Such studies might contribute to the understanding of the role of cAMP PDEs in gametogenesis and in several other processes that are regulated by cAMP.

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