FEBS 15296

FEBS Letters 362 (1995) 131–138

A primordial dopamine D1-like adenylyl cyclase-linked receptor from *Drosophila melanogaster* displaying poor affinity for benzazepines

Kim S. Sugamori[b,d], Lidia L. Demchyshyn[b,d], Fortunata McConkey[d], Michael A. Forte[c], Hyman B. Niznik[a,b,d,*]

[a]*Department of Psychiatry, University of Toronto, Toronto, Ont., M5S 1A8, Canada*
[b]*Department of Pharmacology, University of Toronto, Toronto, Ont., M5S 1A8, Canada*
[c]*Vollum Institute for Advanced Biomedical Research, Portland, OR 97201, USA*
[d]*Laboratory of Molecular Neurobiology, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ont., M5T 1R8, Canada*

Received 20 February 1995

Abstract **We report here the isolation from *Drosophila melanogaster* of a 2.0 kb cDNA clone encoding a 385 amino acid protein (*d*DA1) displaying, within putative transmembrane domains, highest amino acid sequence homology (49–53%) to members of the vertebrate dopamine D1-like receptor family. When expressed in either Sf9 or COS-7 cells, *d*DA1 did not bind the specific D1-like receptor antagonist [^{3}H]SCH-23390 or numerous other dopaminergic, adrenergic or serotoninergic ligands with high affinity. However, like vertebrate dopamine D1-like receptors, *d*DA1 stimulated the accumulation of cAMP in response to DA (EC_{50} ~300 nM) and 6,7-ADTN (EC_{50} ~500 nM). The dopaminergic rank order of potency (DA > NE ≫ 5-HT) and the lack of stimulation by other possible neurotransmitters (octopamine, tyramine, tryptamine) or DA metabolites (e.g. *N*-acetyl dopamine) found in *Drosophila* suggests that this receptor functionally belongs to the dopamine D1-like subfamily. Benzazepines, which characteristically bind to vertebrate dopamine D1-like receptors with high affinity, were relatively poor in stimulating (SKF-38393, SKF-82526; EC_{50} > 10 μM) *d*DA1-mediated accumulation of cAMP. Of the numerous compounds tested, a few dopaminergic antagonists inhibited DA-stimulated production of cAMP in a concentration-dependent manner, albeit with considerably reduced affinity, and with the rank order of potency: (+)-butaclamol(K_b~125nM) > SCH-23390(K_b~230nM) > α-flupenthixol (K_b ~400 nM) > chlorpromazine ≥ spiperone (K_b ~680 nM) ≥ clozapine. In situ hybridization revealed that *d*DA1 receptor mRNA is expressed as a maternal transcript, and at later blastoderm stages is restricted to apical regions of the cortical peripheral cytoplasm. The generation of inter-species D1 receptor chimeras may help to identify those particular sequence-specific motifs or amino acid residues confering high affinity benzazepine receptor interactions.**

Key words: G protein-coupled receptor; Invertebrate; cAMP; Catecholamine

1. Introduction

Dopamine, a neurotransmitter in the nervous system of vertebrates, acts on a number of central and peripheral receptors to exert its physiological and neuromodulator effects [1,2]. Receptors for dopamine have been cloned and characterized from several vertebrate species. These receptors have typically been classified as D1-like (D1, D1B/D5) or D2-like (D2, D3, D4) based on pharmacological, biochemical and structural criteria (for reviews see [3–5]). The recent cloning of the D1C receptor from *Xenopus laevis* [6] and D1D receptor from *Gallus domesticus* [7] confirm that the the D1-like receptor family is as heterogeneous and complex as pharmacological, behavioural and/or biochemical data suggest [8–14].

Dopamine receptors are believed to exist in the invertebrate nervous system; however, the positive identification and classification of such receptors is inconclusive based on the existing pharmacological and biochemical data (for review see [15]). Dopamine is present in relative abundance in the invertebrate CNS, and dopamine-sensitive adenylyl cyclase has been observed in the molluscan and arthropod nervous system [16–19]. Biochemical, electrophysiological and behavioural studies indicate that putative invertebrate dopamine receptors possess pharmacological profiles distinct from mammalian or vertebrate dopamine receptors [15].

Whereas receptors for serotonin and tyramine have been cloned and functionally expressed from *Drosophila* [20–23], dopamine D1- or D2-like receptor genes have not yet been functionally characterized from this species. Dopaminergic neurons and pathways have, in particular, been well mapped by glyoxylic acid-induced histofluorescence and immunohistochemistry in the CNS of *Drosophila melanogaster* [24,25]. A role for dopamine as a neurotransmitter has not been clearly defined in *Drosophila* CNS; however, alterations in the synthesis of dopamine and/or serotonin by mutants genetically deficient in dopa decarboxylase (DDC) appear to result in pertubations of learning [26]. Several well-characterized genetic mutations prominently expressed in the mushroom bodies affect learning and memory, such as *dunce* (a cAMP phosphodiesterase),

*Corresponding author. Fax: (1) (416) 979-4663.

Sequences reported in this paper have been deposited in GenBank with Accession Number U22106.

Abbreviations: (±)-6,7-ADTN, 6,7-dihyroxy-2-aminotetralin; CY 208-243, (−)-4,6,6a,7,8, 12b-hexahydro-7-methyl-indolo[4,3-ab]-phenanthridine; DA, dopamine; DHA, dihydroalprenolol; 8-OH-DPAT, (±)-8-hydroxy-*N,N*-dipropyl-2-aminotetralin; 5-HT, serotonin (5-hydroxytryptamine); L-dopa, L-3,4-dihydroxphenylalanine; LSD, lysergic acid diethylamine; NE, norepinephrine; *N*(−)-NPA, *N*-propyl-norapomorphine; SCH-23390, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SKF-38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine; SKF-81927, 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SKF-82526, 6-chloro-7,8-dihydroxy-1-(*p*-hydroxyphenyl)-2,3,4,5-tetrahydro-(1H)-3-benzazepine; TM, transmembrane; WB-4101, 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane; YM-09151-2, *cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylamino-benzamide.

0014-5793/95/$9.50 © 1995 Federation of European Biochemical Societies. All rights reserved.
SSDI 0014-5793(95)00224-3

rutabaga (a Ca^{2+}/calmodulin-sensitive adenylyl cyclase), and *DCO* (protein kinase A catalytic subunit) [27]. Interestingly, dopaminergic fibers have been found to innervate the mushroom bodies of *Drosophila* [25]. Because *Drosophila* is such an ideal model for genetic manipulations, and since little is known about the pharmacology, structure and functional coupling of dopamine receptors, we initiated a program to isolate dopaminergic D1-like receptors from *Drosophila*.

We report here the cloning of a *Drosophila melanogaster* dopamine D1-like receptor, which, like its vertebrate counterparts, potently stimulates adenylyl cyclase activity. This receptor, however, is pharmacologically distinct from vertebrate D1 receptors, displaying poor affinity for benzazepine ligands. The cloning of a D1-like receptor with low overall homology to vertebrate dopamine D1-like receptors may facilitate the identification of the amino acid residues and/or structural motifs that are conserved throughout D1 receptor evolution and which may impart functional and pharmacological specificity. Furthermore, the presence of this receptor in the *Drosophila* CNS substantiates the view that dopamine may have been well established as a neurotransmitter before the separation of arthropod and chordate lineages [28].

2. Materials and methods

2.1. Isolation of Drosophila genomic and cDNA clone

A *Drosophila melanogaster* adult Canton S strain genomic library (Clontech) was screened with a ^{32}P-labeled human dopamine D5 receptor fragment [29] corresponding to TM domains 2–5 under conditions previously described [6]. Approximately 10^5 independent clones were screened under the following conditions: duplicate nylon filters (NEN/Dupont) were hybridized at 42°C in a solution [30] containing ^{32}P-labeled D5 fragment (2×10^6 cpm/ml). Filters were washed twice in 2 × SSC, 1% SDS for 15 min at 60°C. Upon plaque purification of hybridizing clones, subcloning and Southern blot analysis, a 800 pb *Pst*I fragment was identified by sequence analysis to contain regions showing homology to the first extracellular loop between TM2 and TM3 and the putative TM 3 region of the dopamine D5 receptor. Because an intron was present after TM3, a *Pst*I–*Cla*I 250 bp fragment was labeled with ^{32}P and used to screen an adult head *Drosophila* cDNA library under conditions described above. Four positive clones (1.8–2.0 kb) were isolated and found by restriction mapping and sequencing to be identical to each other and to contain a sequence identical to the partial genomic fragment. Following subcloning into pBluescript SK− (Stratagene), both strands of one clone (*d*DA1) were sequenced using the Sanger dideoxy-chain termination method with 7-deaza-dGTP and Sequenase (USB) with either specific internal primers (Biotechnology Service Centre HSC, Toronto) or T7/T3 primers.

2.2. Cell culture and expression in COS-7 cells

COS-7 cells were grown on 150 mm plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C, 5% CO_2. The *d*DA1 cDNA clone was subcloned into the expression vector PCD-PS for transient expression [31]. COS-7 cells were transfected with the cesium-purified constructs by electroporation as previously described [30].

2.3. Cell culture and expression in Sf9 cells

The *d*DA1 cDNA was cloned in the transfer plasmid pBlueBacIII (Invitrogen). Log phase Sf9 (*Sporodoptera frugiperda*) cells grown in complete Grace's insect media TNM-FH (Gibco BRL) at 27°C in a humid environment were seeded at a cell density of 2×10^6 per 60 mm plate. Cesium-purified pBlueBacIII-*d*DA1 was subsequently co-transfected with linearized wild-type AcMNPV DNA (Invitrogen) using the cationic liposome-mediated transfer essentially as described by the manufacturer. The recombinant virus was purified by several rounds of plaque purification to ensure no wild-type virus was present, and was tested by PCR analysis with internal oligonucleotide primers (5′ oligo, 5′-ATTTTGTGATACTTGGGTGGCCTT-3′; 3′ oligo, 5′-AACTCCTTGTTGAAGATCGAATAG-3′).

2.4. cAMP accumulation

COS-7 cells transiently expressing the *d*DA1 clone and untransfected cells were tested for cAMP accumulation as described previously [6]. For testing stimulation of cAMP production in Sf9 cells grown in Sf900 media (Gibco/BRL), the cells were first split into 24 well plates and infected with the *d*DA1 virus (2×10^6 pfu/well). After 48 h, the wells were washed once with prewarmed Graces' incomplete media containing 0.5 mM 3-isobutyl-1-methylxanthine. Cells were incubated in 0.4 ml of the afore-mentioned medium in the presence or absence of the indicated concentration of antagonist for 15 min at 27°C followed by the addition of agonist for an additional 15 min at 27°C. Reactions were stopped by the addition of 500 μl of 0.2 N HCl, the cellular debris pelleted by centrifugation (500 × *g*) and supernatants (5–10 μl) assayed for cAMP formation by radioimmunoassay (Amersham).

2.5. In situ localization of dDA1 receptor mRNA

Probes for in situ hybridization were generated by random priming to digoxygenin-dUTP of a 350 bp *Xba*I–*Eco*RI fragment representing primarily the 3′ untranslated region of dDA1 cDNA. Collection and processing of embryos for in situ hybridization was essentially as described in Quan et al. [32].

3. Results and discussion

Using a strategy based on homology probing with the dopamine D5 receptor we isolated a partial genomic dopamine D1-like receptor fragment, encoding the first intracellular loop, TM3 and part of the second extracellular loop from *Drosophila melanogaster*. Consensus sequences for 3′ and 5′ splice sites were present in this fragment before and after the small coding region [33]. Due to the presence of introns, a *Pst*I–*Cla*I 250 bp coding fragment was used to screen a *Drosophila* adult head cDNA library. Four cDNA clones were isolated and found to be identical to each other. Sequence analysis of one of these clones indicated that it contained a putative inititiation methionine with predicted Kozak sequence [34] followed by a long open reading frame of 1155 nucleotides encoding a 385 amino acid protein of calculated molecular mass of 43,139 Da.

Hydrophobicity analysis of the deduced amino acid sequence (data not shown) revealed the presence of 7 hydrophobic amino acid segments that form putative transmembrane domains. Comparison of the deduced amino acid sequence of *d*DA1 with other G protein-coupled receptors indicates that the regions of greatest homology occur within these putative transmembrane domains (Fig. 1). The degree of sequence identity within these TM domains was highest with the dopamine D1-like receptor subfamily: 53% to *Xenopus* D1A and D1C receptors; 52% to *Xenopus* D1B; 51% to human D1, chicken D1A and D1D

→

Fig. 1. Deduced amino acid alignment of *Drosophila d*DA1 to cloned members of the dopamine D1-like receptor family. Boxed and shaded areas denote absolutely conserved amino acid residues among all receptors presented. Boxed only regions indicate amino acid residues conserved between *d*DA1 and at least two other dopamine D1-like receptors. Putative transmembrane domains are demarcated above. Consensus sequences for potential protein kinase A (PKA) phosphorylation sites are indicated by filled squares. The single letter amino acid code is used. Sequences sources are as follows: human D1 [31,53,54], monkey D1 [55], rat D1A [53], opposum D1A [56], goldfish D1 [57], *Xen* D1A, D1B and D1C [6], human D5 [29,58,59], rat D1B [60], chicken D1A, D1B, D1D [7].

```
                                                                 TRANSMEMBRANE 1                              TRANSMEMBRANE 2
dDA1 DRO                              MDTIVGEEPEP-LSLVSIVVVGIFLSVLIFLSVAGNILVCLAIYTERSLR-RIGNLFLASLAIADLFVASLVMTFAGVNDLLG-YWIFGA
D1 HUMAN                       MRTLNTSAMDGTGLVVERDFSVRILTACFLSLLILSTLLGNTLVCAAVIRFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAG-FWPFGS
D1 MONKEY                      MRTLNTSAMDGTGLVVERDFSVRILTACFLSLLILSTLLGNTLVCAAVIRFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAG-FWPFGS
D1A RAT                         MAPNTSTMDEAGLPAERDFSFRILTACFLSLLILSTLLGNTLVCAAVIRFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAG-FWPFGS
D1A OPOSSUM                     MPLNDTTMDRRGLVVERDFSFRILTACFLSLLILSTLLGNTLVCAAVIRFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAG-FWPFGS
D1A CHICKEN                     MTWNDTTMDGEGLLVERDSSFRILTGCFLSLLILSTLLGNTLVCAAVIRFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVSEIAG-FWPFGS
D1A FROG                        MTFNITSMDEDVLLTERESSFRVLTGCFLSVLILSTLLGNTLVCAAVIRFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVAEIAG-FWPFGT
D1 GOLDFISH                   MAVLDLNLTTVIDSGFM-ESDRSVRVLTGCFLSVLILSTLLGNTLVCAAVTKFRHLRSKVTNFFVISLAVSDLLVAVLVMPWKAVTEVAG-FWPFGA
D5 HUMAN       MLPPGSNGTAYPGQFALYQQLAQGNAVGGSAGAPPLGPSQVVTACLLTLLIIWTLLGNVLVCAAIVRSRHLRANMTNVFIVSLAVSDLFVALLVMPWKAVAEVAG-YWPFGA
D1B RAT       MLPPGRNRTAQPARLGLQRQLAQVDAPAGSA--TPLGPAQVVTAGLLTLLIVWTLLGNVLVCAAIVRSRHLRAKMTNIFIVSLAVSDLFVALLVMPWKAVAEVAG-YWPFGT
D1B CHICKEN                  MLRGGRSPLPPPAGPPGGARGQAGAGAAQVAAGSLLALLILWTLFGNVLVCAAIVRYRHLRSKVTNIFIVSLAVSDLLVAVLVMPWKAVAEVAG-YWPFGA
D1B FROG     MYQPFQHLDSDQVASWQSPEMLMNKSVSRESQRRKELVAGQIVTGSLLLLLIFWTLFGNILVCTAVMRFRHLRSRVTNIFIVSLAVSDLLVALLVMPWKAVAEVAG-HWPFGA
D1C FROG                 MENFSIFNVTVNVWHADLDVGNSDLSLRALTGLLLSLLILSTLLGNTLVCLAVIKFRHLRSKVTNFFVISLAVSDLFVALLVMPWKAVTEVAG-FWVFGD
D1D CHICKEN                                MASMAGGPRALRALTGCLLGALVLGTLAGNALVCLAVLRFRHLRTKVTNWFVLSLAISDLCVAILVMPWKAVTEVAGGFWLFGS

                 TRANSMEMBRANE 3                      TRANSMEMBRANE 4
dDA1 DRO     QFCDTWVAFDVMCSTASILNLCAISMDRYIHIKDPLRYGRWVTRRVAVITIAAIWLLAAFVSFVPISLGIHRPDQPLIF-EDNGKKYPT---------------------------
D1 HUMAN     -FCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTPKAAFILISVAWTLSVLISFIPVQLSWHKAK-PTSPS-D-G----NATSLAE--------NIDN-------
D1 MONKEY    -FCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTPKAAFILISVAWTLSVLISFIPVQLSWHKAK-PTSPS-D-G----NATSLAE--------TIDN-------
D1A RAT      -FCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFQYERKMTPKAAFILISVAWTLSVLISFIPVQLSWHKAK-PTWPL-D-G----NFTSL-ED-----TEDDN-------
D1A OPOSSUM  -FCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTPKAAFILISVAWTLSVLISFIPVQLNWHKAR-PLSSP-D-G----NVSSQDET--------MDN-------
D1A CHICKEN  -FCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTPKAAFIMISVAWTLSVLISFIPVQLNWHKAT-TTSFL-DL-----NASLQGIS--------MDN-------
D1A FROG     -FCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTPKVAFIMIGVAWTLSVLISFIPVQLNWHKAK-TTSFF-DL-----NIT-LH-D-----RTMDN-------
D1 GOLDFISH  -FCDIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTPRVAFVMISGAWTLSVLISFIPVQLKWHKAQ-PIGFLE--V----NASRRDL--------PTDN-------
D5 HUMAN     -FCDVWVAFDIMCSTASILNLCVISVDRYWAISRPFRYKRKMTQRMALVMVGLAWTLSILISFIPVQLNWHRDQAASWGGLDLPNNLANWTPWEEDFWEPDVNAEN-------
D1B RAT      -FCDIWVAFDIMCSTASILNLCIISVDRYWAISRPFRYERKMTQRVALVMVGLAWTLSILISFIPVQLNWHRDKAGSQGQEGL---LSNGTPWEEF-WELEGRTEN-------
D1B CHICKEN  -FCNVWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTQRLALVMIGVAWALSVLISFIPVQLNWHRGGDAATAAAAGDIEDGFDTGWEAAGAFTTWAEDMSTTWVA
D1B FROG     -FCDIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYERKMTQRVALLMISTAWALSVLISFIPVQLSWHKS-ET---EDH----LSNHS--------------TGN-------
D1C FROG     -FCDTWVAFDIMCSTASILNLCIISLDRYWAIASPFRYERKMTQRVAFIMIGVAWTLSILISFIPVQLSWHKSHEA---DEELVGN--NHT--------------EN-------
D1D CHICKEN  RFCDTWVAFDIMCSTASILHLCIISLDRYWAIASPFRYERKMTQRLACAMIAMAWALSILISFVPVQLHWHKAKFDRRHPSSS--------------------------------

                                             TRANSMEMBRANE 5
dDA1 DRO     --------------------------CALDLTPTYAVVSSCISFYFPCVVMIGIYCRLYCYAQKHVKSIKAVTRPGEVAEKQRYKSIRRPKNQPKKFKVRNLHTHSSPYHVSDH
D1 HUMAN     --------------------------CDSSLSRTYAISSSVISFYIPVAIMIVTYTRIYRIAQKQIRRIAALERAAVHAKNCQTTTGNGKPVECSQPESSFKM---S--FKRET
D1 MONKEY    --------------------------CDSSLSRTYAISSSVISFYIPVAIMIVTYTRIYRIAQKQIRRIAALERAAVHAKNCQTTTGNGKPVECSQPESSFKM---S--FKRET
D1A RAT      --------------------------CDTRLSRTYAISSSLISFYIPVAIMIVTYTSIYRIAQKQIRRISALERAAVHAKNCQTTAGNGNPVECAQSESSFKM---S--FKRET
D1A OPOSSUM  --------------------------CDSSLSRTYAISSSLISFYIPVAIMIVTYTRIYRIAQKQIRRISALERAAVHAKNCQNTTGNGANVECSQPESSFKM---S--FKRET
D1A CHICKEN  --------------------------CDSSLNRMYAISSSLISFYIPVAIMIVTYTRIYRIAQKQIRRISALERAAVHAKNCQNTSGNRSSMDCQQPESNFKM---S--FKRET
D1A FROG     --------------------------CDSSLNRTYAISSSLISFYIPVAIMIVTYTRIYRIAAKQIRRISALERAAVHAKNCQNSTSNRNSLDCQQPESSLKT---S--FKRET
D1 GOLDFISH  --------------------------CDSSLNRTYAISSSLISFYIPVAIMIVTYTQIYRIAQKQIRRISALERAAESAQIRHDSMGSGSNMD---LESSFKL---S--FKRET
D5 HUMAN     --------------------------CDSLLNRTYAISSSLISFYIPVAIMIVTYTRIYRIAQVQIRRISSLERAAEHAQSCRSSAA-------C-APDTSLRA---S--IKKET
D1B RAT      --------------------------CDSSLNRTYAISSSLISFYIPVAIMIVTYTRIYRIAQVQIRRISSLERAAEHAQSCRSRGA-------Y-EPDPSLRA---S--IKKET
D1B CHICKEN  LAAMTPSEGTSGTSGSNNTVPSESCDSSLNRTYAISSSLISFYIPVAIMIVTYTRIYRIAQVQIRRISSLERAAEHAQSCRCNHVD-C------HHTSLK---S--IRKET
D1B FROG     --------------------------CDSSLNRTYAISSSLISFYIPVAIMIVTYTRIYRIAQIQIKRISTLERAAEHAQSCRSNRVDSC---SRHHQTSLRT---S--IKKET
D1C FROG     --------------------------CDSSLNRTYAISSSLISFYIPVVIMIGTYTRIYRIAQTQIRRISSLERAVEHAQRCSSRLSN---------ENSLKT---S--FRKET
D1D CHICKEN  --------------------------CDVSLNRTYAITSSLISFYIPVAIMIVTYTRIYRIAQAQIRRISTLERAGGQWPAHSKEPA-----------STLRS---S--LRKET

                 TRANSMEMBRANE 6                           TRANSMEMBRANE 7
dDA1 DRO     KAAVTVGVIMGVFLICWVPFFCVNITAAFC--------KT---CIGGQTFKILTWLGYSNSAFNPIIYSIFNKEFRDAFKRILTMRNPWCCAQDVGNIHPRNSDRFITDYAA
D1 HUMAN     KVLKTLSVIMGVFVCCWLPFFILNCILPFCG-----SGETQPFCIDSNTFDVFVWFGWANSSLNPIIYA-FNADFRKAF-STLLGCYRLCPATNNA-IETV--SINNNGAAM
D1 MONKEY    KVLKTLSVIMGVFVCCWLPFFILNCILPFCG-----SGETQPFCIDSITFDVFVWFGWANSSLNPIIYA-FNADFRKAF-STLLGCYRLCPATNNA-IETV--SINNNGAAM
D1A RAT      KVLKTLSVIMGVFVCCWLPFFISNCMVPFCG-----SEETQPFCIDSITFDVFVWFGWANSSLNPIIYA-FNADFQKAF-STLLGCYRLCPTTNNA-IETV--SINNNGAVV
D1A OPOSSUM  KVLKTLSVIMGVFVCCWLPFFILNCMVPFCESDLP-SGETKPFCIDSITFDVFVWFGWANSSLNPIIYA-FNADFRKAF-STLLGCYRLCPTANNA-IETV--SINNNGAV-
D1A CHICKEN  KVLKTLSVIMGVFVCCWLPFFVLNCMIPFCEPTQP-SKGAEAFCINSTTFDVFIWFGWANSSLNPIIYA-FNADFRKAF-STLLGCYRLCPMSGNA-IETV--SINNNGAVV
D1A FROG     KVLKTLSVIMGVFVCCWLPFFILNCIVPFCDPSLTTSG-TEPFCISSTTFDVFVWFGWANSSLNPIIYA-FNADFRKAF-SNLLGCYRLCPTSNNI-IETV--SINNNGAVV
D1 GOLDFISH  KVLKTLSVIMGVFVCCWLPFFILNCMVPFC---KRTS--NGLPCISPTTFDVFVWFGWANSSLNPIIYA-FNADFRRAF-AILLGCQRLCPGSIS---ETP--SLNKN 363
D5 HUMAN     KVLKTLSVIMGVFVCCWLPFFILNCMVPFCS-GHPEGPPAGFPCVSETTFDVFVWFGWANSSLNPVIYA-FNADFQKVF-AQLLGCSHFCSRTP---VETVNISNELISYNQ
D1B RAT      KVFKTLSMIMGVFVCCWLPFFILNCMVPFCSSGDAEGPKTGFPCVSETTFDIFVWFGWANSSLNPIIYA-FNADFRKVF-AQLLGCSHFCFRTP---VQTVNISNELISYHQ
D1B CHICKEN  KVLKTLSIIMGVFVCCWLPFFILNCMVPFCESP-PSDPRAGLPCVSETTFNIFVWFGWANSSLNPIIYA-FNADFRKVF-SNLLGCGQFCSSTP---VETVNISNELISYHQ
D1B FROG     KVLKTLSIIMGVFVCCWLPFFILNCMVPFCDRS-PGHPQAGLPCVSETTFDIFVWFGWANSSLNPIIYA-FNADFRKVF-SSLLGCGHWCSTTP---VETVNISNELISYNQ
D1C FROG     KVLKTLSIIMGVFVFCWLPFFVLNCMIPFCHMNLPGQNEPEPPCVSETTFNIFVWFGWANSSLNPVIYA-FNADFRKAF-TTILGCNRFCSS-NN--VEAVNFSNELVSYHH
D1D CHICKEN  KVLQTLSIIMGVFVCCWLPFFILNCLLPFCQPESD-SNG-QSPCVGQTTFNVFVWFGWANSSVNPVIYA FNADFRRAF-SNLLGCQRLCCSTPRAAVERVNLSNELVSYHQ

dDA1 DRO     KNVVVMNSGRSSAELEQVSAI   385
D1 HUMAN     FSSHHEPRGSISKECNLVYLIPHAVGSSED--LKKEEAAGIA---RPLEKLS-PALSVI-LDYDTDVSLEKIQPITQNGQHPT   446
D1 MONKEY    FSSHHEPRGSISKECNLVYLIPHAVGSSED--LKKEEAAGIA---RPLEKLS-PALSVI-LDYDTDVSLEKIQPITQNGQHPT   446
D1A RAT      FSSHHEPRGSISKDCNLVYLIPHAVGSSED--LKKEEAGGIA---KPLEKLS-PALSVI-LDYDTDVSLEKIQPVTHSGQHST   446
D1A OPOSSUM  FSSHHEPRGSISKDCNLVYLIPQAV-TSRD--PKKEEGG-S---KPLEKTS-PALSVI-LDYEVDLSLEKINPITQNGQHYT   446
D1A CHICKEN  FSSQHEPKGSSPKESNLVYLIPHAIICPEEEPLKKEEEGELS---KTLEKMS-PALSGI-LDYEADVSLEKINPITQNGQHKT   451
D1A FROG     YSCQQEPKGSIPNECNLVYLIPHAIICPEDEVLKKEDESGLS---KSLEKMS-PAFSGI-LDYDADVSLEKINPITQNGQPKT   451
D5 HUMAN     DIVFHKEIAAAYIH-MMPNAVTPGNREVDNDEEEGPFDRMFQIYQTSPDGDPVAE-SVW-LDCEGEISLDKITPFTPNGFH   477
D1B RAT      DTVFHKEIATAYVH-MIPNAVSSGDREVGEEEEEGPFDHMSQISPTTPDGDLAAE-SVWELDCEEEVSLGKISPLTPNCFDKTA   475
D1B CHICKEN  DTLFHKEIVTAYVN MIPNVVDCEENRED------PFDRMSQI---SPDPEVATD-SVCELDCEGEISLGKITPFTPNGLH   487
D1B FROG     DTLFHKDIVTAYVN-MIPNVVDC-ID--DNED---AFDHMSQISQTSANNELATD-SMCELDSEVDISLHKITPSMSNGIH   457
D1C FROG     DTTFQKDIPVTFNNSHLPNVVDQDQEVLEGTCFDKVSVLSTSHGTRSQKNLHLPA-GVQF-ECEAEITLETITPFTSTGPLECLPQLVADEDRHYTTKLY   465
D1D CHICKEN  DTTCQKDGATAAIPPTTVTAWVQPEPHVVSPQQEDREELPTEERPVAAPTQAMPGSSPKCCHVTATSQLDCGADLSLETINAFTGFSRHEGHHHPIPEG   445
```

receptors; and 49% to human D5 and chicken D1B receptors. *d*DA1 sequence identity within TM domains to other biogenic amine G-linked receptors is: 46% to the human dopamine D2L; 45% to human $\beta1$ and $\beta3$ ARs, *Drosophila* 5HTdro1 and tyramine; 44% to human $\beta2$ AR and dopamine D4; 43% to human dopamine D3 and $\alpha1$A AR; and 41% to human $\alpha2$ AR. Overall amino acid sequence identities are considerably lower. *d*DA1 displays 22% amino acid sequence identity with the 5HTdro1 and tyramine receptors; 26% to human dopamine D2L and D3 receptors; 27% to human $\alpha1$A and $\beta2$ AR; 28% to human $\alpha2$ AR, $\beta1$ AR, and dopamine D4 receptors and 30% to $\beta3$ AR. The *d*DA1 receptor appears to display a slightly higher degree of overall sequence homology to vertebrate dopamine D1-like receptors (29% to human dopamine D1, chicken D1A and D1B receptors, 30% to *Xenopus* dopamine D1A and D1C, 31% to human D5 and chicken D1D receptors, and 33% to *Xenopus* dopamine D1B receptor).

Conserved in *d*DA1 are the aspartic residue (Asp^{97}) in TM3, two cysteine residues that may partake in disulfide bond formation between the second and third extracellular loops [35], and two serine (Ser^{189}; Ser^{192}) residues in TM5 that may form part of the dopamine binding pocket [36]. No putative sites for N-linked glycosylation sites are apparent in the amino-terminus or second extracellular loop where almost all vertebrate dopamine D1 receptors have one N-linked glycosylation site. One conserved putative protein kinase C site is found in the second cytoplasmic loop (T130) while several other non-conserved putative protein kinase C sites are found in the third cytoplasmic loop (S^{217}, S^{235}) and carboxy-tail (T^{336}, S^{355}, S^{372}). Thus far, the presence of these putative phosphorylation sites within the carboxy-tail has been unique to only the *Xenopus* D1C and chicken D1D receptors. The functional significance of these sites is at present unknown but may play a role in receptor desensitization.

Other rather unique structural features of *d*DA1 include a very short extracellular loop between TM6 and TM7 and the relatively short carboxy-tail. Moreover, sequences in the third cytoplamic loop and intracellular carboxy-tail do not show strong sequence conservation with vertebrate D1-like receptors. Minigene and mutagenesis studies indicate that the third cytoplasmic loop is important for mediating receptor coupling to subtype-specific G proteins [37–40]. Thus, *d*DA1 lacks the BBAXB (B = base; A = acid; X = any residue) motif at the carboxyl-end of the third cytoplasmic loop which is conserved in all D1-like receptors and has been implicated as one of the regions regulating G_s protein activation. Interestingly enough, this sequence motif may be inverted in *d*DA1 (BXXABB). Other putative G protein-activating motifs, BBXB, [41] are, however, present in both the amino (KHVK) and middle (RRPK; KKFK) portions of this loop. In addition to the third cytoplasmic loop, sequences within the carboxyl-tail have been shown to determine G protein specificity [42]. In any event, the lack of a high degree of amino acid correspondence suggests that *d*DA1 is evolutionarily so far removed from its vertebrate counterparts that it can not be reliably classified as a member of the D1 subfamily of receptors based on structure alone.

Table 1
Lack of detectable specific receptor binding activity in COS-7 and Sf9 cell membranes expressing the *d*DA1 receptor

Labeled compound		Receptor selectivity	Displacer
COS-7 cells			
[³H]SCH-23390	(5.0 nM)	Dopamine D1 receptors	100 μM DA 10 μM SKF-82526 10 μM (+)-butaclamol 10 μM α-flupenthixol 5 μM SCH-23390
[³H]Spiperone	(2.3 nM)	Dopamine D2 receptors	10 μM (+)-butaclamol
[³H]α-Flupenthixol	(3.6 nM)	Dopamine D1 receptors	5 μM SCH-23390
[³H]DHA	(5.0 nM)	β-Adrenergic receptors	10 μM propranolol
[³H]Prazosin	(6.2 nM)	α-Adrenergic receptors	1 μM prazosin
[³H]Yohimbine	(4.8 nM)	α-Adrenergic receptors	10 μM WB-4101
[³H]LSD	(2.4 nM)	Non-selective 5-HT	10 μM methiothepin
[³H]5-HT	(5.0 nM)	5-HT1 receptors	100 μM 5-HT
[³H]8-OH-DPAT	(5.5 nM)	5-HT1A receptor	100 μM 5-HT
Sf9 cells			
[³H]SCH-23390	(3.3 nM)	Dopamine D1 receptors	50 μM α-flupenthixol 100 μM NE, 100 μM tryptamine
[³H]Spiperone	(1.8 nM)	Dopamine D2 receptors	100 μM tryptamine
[³H]Raclopride	(4.8 nM)	Dopamine D2 receptors	100 μM tryptamine
[³H]YM-09151-2	(1.6 nM)	Dopamine D2 receptors	100 μM tryptamine
[³H]Tryptamine	(9.4 nM)	–	100 μM tryptamine
[³H]Prazosin	(6.6 nM)	α-Adrenergic receptors	100 μM DA/NE/5-HT
[³H]Yohimbine	(12.1 nM)	α-Adrenergic receptors	100 μM DA/NE/5-HT
[³H]DHA	(1.0 nM)	β-Adrenergic receptors	100 μM DA/NE/5-HT
[³H]WB-4101	(12.0 nM)	α-Adrenergic/5-HT1A receptors	100 μM DA/NE/5-HT
[³H]LSD	(2.3 nM)	Non-selective 5-HT	100 μM DA/NE/5-HT

COS-7 or Sf9 membranes expressing dDA1 receptors were prepared and assayed for radioligand binding to distinct receptor subtypes as described in section 2. No specific binding activity, relative to mock infected cells, was noted under any of the conditions listed. Specific activities (1 Ci = 37 Gbq): [³H]SCH-23390 (NEN, 85.5 Ci/mmol), [³H]spiperone (NEN, 24 Ci/mmol), [³H]α-flupenthixol (NEN, 14.1 Ci/mmol), [³H]YM-09151–2 (NEN, 81.4 Ci/mmol), [³H]raclopride (NEN, 77.0 Ci/mmol), [³H]prazosin (NEN, 82.0 Ci/mmol), [³H]yohimbine (NEN, 70.5 Ci/mmol), [³H]DHA (NEN, 105.5 Ci/mmol), [³H]5HT (NEN, 23.7 Ci/mmol), [³H]8-OH-DPAT (NEN, 163.0 Ci/mmol), [³H]LSD (NEN, 65.2 Ci/mmol), [³H]tryptamine (NEN, 21.2 Ci/mmol).

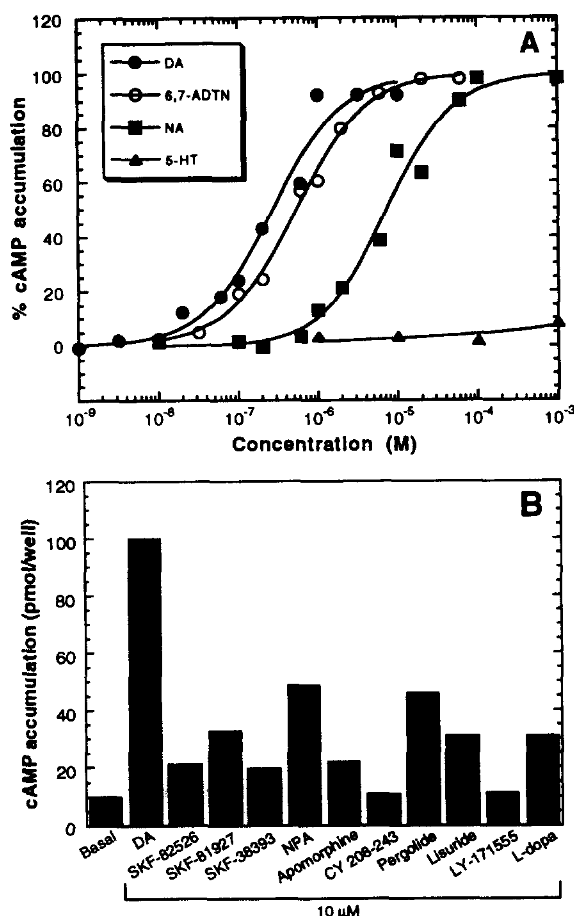

Fig. 2. Pharmacological specificity of cAMP accumulation in Sf9 cells expressing the *d*DA1 receptor. (A) Agonist dose–response curves for *d*DA1-mediated cAMP accumulation. Infected Sf9 cells were incubated with increasing concentrations of the indicated agonist, and the amount of cAMP measured as described in section 2. Estimated EC_{50} values were determined by KALEIDAGRAPH and are representative of two independent experiments conducted in duplicated and which varied by less than 15%. (B) Bar graph depicting levels of cAMP accumulation produced by various dopaminergic agonists (10 μM) compared to basal levels and 10 μM DA.

In order to accurately determine the pharmacological identity of *d*DA1 we assessed the ability of a number of radiolabeled compounds to detect receptor activity on membranes prepared from COS-7 cells transiently expressing the *d*DA1 receptor. As listed in Table 1, no specific binding was obtained with tritiated ligands commonly used to define dopamine D1-, D2-like receptors, β- and α-adrenergic receptors or 5-HT receptors. Most significantly, the benzazepine SCH-23390 which binds to D1-like receptors with high affinity did not bind to the expressed *d*DA1 receptor. Similarly, using a baculovirus expression system which allows for the high expression of proteins, no specific binding was obtained on membranes prepared from Sf9 cells infected with the *d*DA1 receptor. The lack of *d*DA1 binding with conventional ligands, which normally act as selective, high affinity antagonists at mammalian or vertebrate D1-like receptors is not surprising. The *Drosophila* 5HT1, 5HT2A, and 5HT2B [20–23] and *Lymnaea* 5HTlym [43] receptors, in particular, display pharmacological profiles distinct from their vertebrate counterparts and are insensitive to commonly used tritiated ligands used for serotonin receptor identification. These data, when taken together, suggests that the acquisition of these binding sites, particularly that for antagonists, appeared to have developed some time after the divergence of invertebrates and vertebrates.

Since dopamine-stimulated cAMP production has been observed in invertebrates, including cockroach, locust, molluscs and planaria [17–19,44] we assessed the ability of *d*DA1 to stimulate the formation of cAMP in both COS-7 and Sf9 cells. In COS-7 cells transiently expressing *d*DA1, 10 μM DA, 100 μM SKF-82526, 100 μM NPA, and 50 μM apomorphine stimulated the production of cAMP, albeit to varying levels (3–5 fold). The *d*DA1 receptor was also tested for its ability to stimulate adenylyl cyclase in Sf9 cells. This system may in fact be more appropriate for the assessment of *d*DA1-mediated functional responsivity since the complement of G proteins and

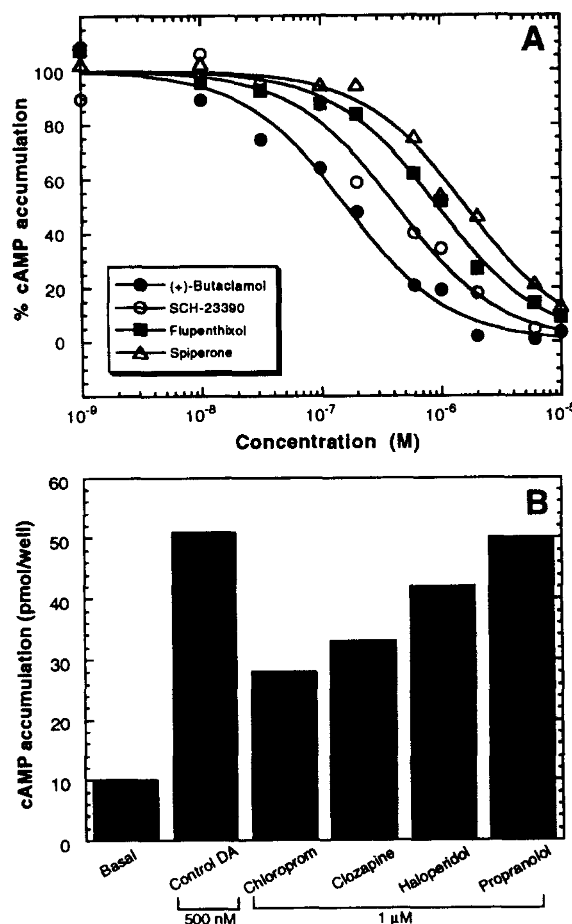

Fig. 3. Pharmacological specificity of the inhibition of DA-stimulated cAMP accumulation is Sf9 cells expressing *d*DA1 receptor. (A) Inhibition of DA-stimulated accumulation of cAMP by dopaminergic antagonists. Infected Sf9 cells were pretreated for 15 min with increasing concentrations of the indicated antagonists followed by addition of 500 nM DA (Af) for an additional 15 min incubation period. Estimated IC_{50} and K_b values were determined by KALEIDAGRAPH and a modified Cheung-Prussoff equation [$K_b = IC_{50'}/([A_f]/EC_{50'}) - 1$], respectively, where Af represents the fixed concentration of dopamine used as described [45]. Data shown are representative of at least two independent experiments each conducted in duplicate and which varied by less than 15%. (B) Bar graph illustrating the ability of various antagonists (1 μM) to inhibit levels of cAMP accumulation generated by the addition of 500 nM DA.

Fig. 4. In situ localization of *d*DA1 receptor mRNA to *Drosophila* embryos. Figure depicts developmental labeling pattern of *d*DA1 receptor mRNA during (A) syncitial stage embryo, (B) cellular blastoderm stage embryo, and (C) germ band extension stage embryos. Anterior is to the left, posterior is to the right. A random primed digoxygenin-dUTP 350 bp *Xba*I–*Eco*RI fragment representing primarily the 3′ untranslated region of *d*DA1 cDNA was used as a probe with collection and processing of embryos for in situ hybridization as described in section 2.

adenylyl cylase enzymes present in Sf9 cells may be more representative of what is constitutive in native neural *Drosophila* cells. In fact, there was an enhanced stimulation of adenylyl cyclase activity by dopamine (~10 fold), as determined by the increased levels of cAMP accumulation, relative to that seen with COS-7 cells (data not shown). As depicted in Fig. 2A, dopamine and ADTN stimulated, in a concentration-dependent manner, the production of cAMP in *d*DA1-infected Sf9 cells with high affinity (EC_{50} 300–500 nM, respectively) relative to NE (EC_{50} ~7000 nM) and 5-HT (> 1 mM); a pharmacological profile clearly indicative of a dopamine receptor. As illustrated in Fig. 2B, other dopaminergic agonists or D1 selective agonists (e.g. SKF-82526, NPA, apomorphine, pergolide and L-dopa) also stimulated the production of cAMP, albeit to levels considerable lower than the agonists mentioned above (2–5 fold over basal) with estimated EC_{50} values of 10 μM or higher. Whether these compounds act as low affinity partial agonists at the *d*DA1 receptor is at present unknown. There was no endogenous stimulation of cAMP production by these agonists as determined in mock-transfected Sf9 cells tested in parallel (data not shown). The selective dopamine D2-like receptor agonist LY-17555 and non-selective D1:D2 receptor agent CY-208–243 were ineffective (Fig. 2B). Similarly other neurotransmitters and agents, such as tyramine, octopamine, serotonin, tryptamine, *N*-acetyl DA and isoproterenol (at concentrations of up to 10 μM each) were ineffective in stimulating the production of cAMP (data not shown).

As illustrated in Fig. 3A, the pharmacological profile of classically defined dopamine receptor antagonists at inhibiting *d*DA1-mediated stimulation of cAMP in Sf9 cells is distinct from that of the vertebrate D1-like receptor family. Thus, dopamine (500 nM)-mediated *d*DA1 stimulation of cAMP was inhibited in a competitive and concentration-dependent manner by a number of dopamine receptor antagonists with the following rank order of potency: butaclamol > SCH-23390 > flupenthixol > spiperone and with estimated K_b values [45] from ~10–100 fold greater than that seen for cloned vertebrate D1-like receptors. The poor affinity displayed by the selective D1 receptor antagonist of the benzazepine class, SCH-23390 (K_b ~230 nM), for *d*DA1-stimulated cAMP is consistent with the notion that, as opposed to vertebrates, invertebrate dopamine receptors are insensitive to this class of molecule (also see above). Other dopamine receptor antagonists, but not βAR antagonists, inhibited *d*DA1-stimulated adenylyl cyclase activity in Sf9 cells, albeit with estimated IC_{50} values ≥ 1 μM (Fig. 3B).

The rank order of potency for neurotransmitter agonists in stimulating cAMP production (DA > NE > 5-HT) and the lack of stimulation by other possible transmitters (octopamine, tyramine, tryptamine) and DA metabolites (*N*-acetyl DA) suggests that this receptor operationally may be defined as a dopamine D1-like receptor. The low homology displayed by *d*DA1 to vertebrate dopamine D1 receptors and the presence of introns suggests that this receptor may be a progenitor of the dopamine D1 receptor subfamily. The fact that selective D1 receptor antagonists (thiothixenes, benzazepines) are relatively poor inhibitors of DA-stimulated cAMP production lends support to the observed presence of a low affinity benzazepine dopamine D1-like receptor stimulating adenylyl cyclase activity in vertebrate species [46]. If so, the *d*DA1 receptor may be a useful probe with which to screen for such a gene in vertebrates. Indeed, Southern blot analysis of digested *Gallus domesticus* DNA depicts one distinct ~700 bp *d*DA1 hydrizing band which is not similarly identified by chicken D1A, D1B and D1D receptor probes (data not shown). Whether this DNA fragment represents a novel dopamine D1-like receptor or another catecholaminergic receptor gene is currently under investigation.

Drosophila is an ideal developmental and genetic model that may be used to study the effects of this receptor on the developing nervous system. Dopaminergic cell clusters appear as early as 18–20 h of *Drosophila* embryonic development [24], and vertebrate dopamine D1-like receptors have been shown to modulate neuronal growth and morphogenesis [47–49]. The spatial and temporal pattern of *d*DA1 receptor mRNA expression during *Drosophila* development was assessed by in situ hybridization to fly embryos with probes encoding 3′ *d*DA1 receptor untranslated regions. *d*DA1 appears to be expressed as a maternal transcript since abundant hybridization is observed at early, syncitial stages prior to the activation of zygotic nuclear transcriptions (Fig. 4A). At these and later cellular blastoderm stages (Fig. 4B), hybridization is restricted to apical regions of the cortical, peripheral cytoplasm. Similar patterns of transcript localization have been observed for several of the *Drosophila* 'pair rule' genes which direct embryonic pattern formation [50]. In these cases, and perhaps for *d*DA1, apical localization of transcripts directs apical compartmentalization of protein products, thereby restricting lateral protein diffusion and allowing for the definition of precise spatial domains. At later stages of development, *d*DA1 transcripts are specifically associated with the extending germ band and excluded from the invaginating posterior mesoderm and presumptive head regions (Fig. 4C). *d*DA1 transcripts appear to be uniformly expressed in all developing tissues at all subsequent stages of embryogenesis (data not shown). Despite these receptor

mRNA localization patterns it is still unclear whether dopamine D1-like receptors affect neuronal differentiation in either *Drosophila* or vertebrates. While still in early stages of characterization, mice deficient in the D1A receptor gene appear to display normal patterns of neuronal circuitry [51]. A recent report, however, has described the genomic organization of the *d*DA1 receptor gene and assigned its localization to the region of chromosome 35 E–F, an area containing a number of mapped lethal mutations (see [52] and refs. therein). It may therefore be important to analyze these mutants in order to gain a better understanding of the regulation of D1-like receptor gene expression and function during *Drosophila* nervous system development.

In summary, the cloning of a possible primordial dopamine D1-like receptor stimulating adenylyl cyclase activity in *Drosophila* may hopefully aid in the identification of those sequence-specific motifs that determine subtype specific D1 receptor G protein coupling and pharmacological activation. Moreover, by the creation of inter-species receptor chimeras, the molecular substrate underlying high affinity benzazepine receptor interactions, which so typifies vertebrate dopamine D1 receptors, may be localized to a defined region or regions of the receptor.

Acknowledgements: The authors wish to thank S. Hamadanizadeh, M. Chung, H.-C. Guan, and P. Seeman for excellent technical advice and assistance. This work was supported in part by grants from the Medical Research Council of Canada (PG-11121), the Ontario Friends of Schizophrenia and the Ontario Mental Health Foundation. K.S.S. is a recipient of a Medical Research Council studentship, and L.L.D. is supported by an Ontario Mental Health Foundation studentship. H.B.N. was supported by a National Association for Research on Schizophrenia and Depression Established Investigator Award, and is a Career Scientist of the Ontario Ministry of Health.

References

[1] Civelli, O., Bunzow, J.R. and Grandy, D.K. (1993) Annu. Rev. Pharmacol. Toxicol. 32, 281–307.
[2] Gingrich, J.A. and Caron, M.G. (1993) Annu. Rev. Neurosci. 16, 299–321.
[3] Niznik, H.B. and Van Tol, H.H.M. (1992) J. Psychiatr. Neurosci. 17, 158–180.
[4] Sibley, D.R. and Monsma Jr, F.J.. (1992) Trends Pharmacol. Sci. 13, 61–69.
[5] Hall, H. (1994) in: Dopamine Receptors and Transporters (Niznik, H.B. ed.) pp. 3–35, Marcel Dekker, New York.
[6] Sugamori, K.S., Demchyshyn, L.L., Chung, M. and Niznik, H.B. (1994) Proc. Natl. Acad. Sci. USA 91, 10536–10540.
[7] Demchyshyn, L.L.,Sugamori, K.S., Lee, F.J.S., Hamadanizadeh, S.A. and Niznik, H.B. (1995) J. Biol. Chem. 270, 4005–4012.
[8] Mahan, L.C., Burch, R.M., Monsma Jr, F.J. and Sibley, D.R. (1990) Proc. Natl. Acad. Sci. USA 87, 2196–2200.
[9] Undie, A.S., Weinstock, J. and Friedman, E (1994) J. Neurochem. 62, 2045–2048.
[10] Laitinen, J.T. (1993) J. Neurochem. 61, 1461–1469.
[11] Mailman, R.B., Shulz, D.W., Kilts, C.D., Lewis, M.H., Rollema, H. and Wyrick, S. (1986) Psychopharmacol. Bull. 22, 593–598.
[12] Arnt, J., Hyttel, J. and Sanchez, C. (1992) Eur. J. Pharmacol. 213, 259–267.
[13] Daly, S.A. and Waddington, J.L. (1993) Psychopharmacol. 113, 45–50.
[14] Downes, R.P. and Waddington, J.L. (1993) Eur. J. Pharmacol. 234, 135–136.
[15] Sugamori, K.S., Van Tol, H.H.M. and Niznik, H.B. (1994) in: Dopamine Receptors and Transporters (Niznik, H.B. ed.) pp. 103–129, Marcel Dekker, New York.
[16] Weiss, S. and Drummond, G.I. (1981) Mol. Pharmacol. 20, 592–597.
[17] Sonetti, D., Biondi, C., Ferretti, M.E., Portolan, A. and Brunelli, M. (1987) Neurochem. Int. 11, 119–126.
[18] Venturini, G. (1993) Comp. Biochem. Physiol. 105C, 297–301.
[19] Ali, D.W. and Orchard, I. (1994) Biogenic Amines 10, 195–212.
[20] Witz, P., Amlaiky, N., Plassat, J.-L., Maroteaux, L., Borrelli, E. and Hen, R. (1990) Proc. Natl. Acad. Sci. USA 87, 8940–8944.
[21] Saudou, F., Boschert, U., Amlaiky, N., Plassat, J.-L. and Hen, R. (1992) EMBO J. 11, 7–17.
[22] Saudou, F., Amlaiky, N., Plassat, J.-L., Borelli, E. and Hen, R. (1990) EMBO J. 9, 3611–3617.
[23] Arakawa, S., Gocayne, J.D., McCombie, W.R., Urquhart, D.A., Hall, L.M., Fraser, D.A. and Venter, J.C. (1990) Neuron 2, 343–354 .
[24] Budnik, V. and White, K. (1988) J. Comp. Neurol. 268, 400–413.
[25] Nassel, D.R. and Elekes, K. (1992) Cell Tissue Res. 267, 147–167.
[26] Tempel, B.L., Livingstone, M.S. and Quinn, W.G. (1984) Proc. Natl. Acad. Sci. USA 81, 3577–3581.
[27] Davis, R.L. (1993) Neuron 11, 1–14 .
[28] Fryxell, K.J. (1994) in: Dopamine Receptors and Transporters (Niznik, H.B. ed.) pp. 237–263, Marcel Dekker, New York.
[29] Sunahara, R.K., Guan, H.-C., O'Dowd, B.F., Seeman, P., Laurier, L.G., Ng, G., George, S.R., Torchia, J., Van Tol, H.H.M. and Niznik, H.B. (1991) Nature 350, 614–619.
[30] Pristupa, Z.B., Wilson, J.M., Hoffman, B.J., Kish, S.J. and Niznik, H.B. (1994) Mol. Pharmacol. 45, 125–135 .
[31] Sunahara, R.K., Niznik, H.B., Weiner, D.M., Stormann, T.M., Brann, M.R., Kennedy, J.L., Gelernter, J.E., Rozmahel, R., Yang, Y., Israel, Y., Seeman, P. and O'Dowd, B.F. (1990) Nature 347, 80–83.
[32] Quan, F., Wolfgang, W.J. and Forte, M.A. (1993) Proc. Natl. Acad. Sci. USA 90, 4236–4240.
[33] Mount, S.M., Burks, C., Hertz, G., Stormo, G.D., White, O. and Fields, C. (1992) Nucleic Acids Res. 20, 4255–4262.
[34] Kozak, M. (1986) Cell 44, 283–292.
[35] Dixon, R.A.F., Sigal, I.S., Candelore, M.R., Register, R.B., W. Scattergood,W., Rands, W. and Strader, C.D. (1987) EMBO J. 6, 3269–3275.
[36] Pollock, N.J., Manelli, A.M., Hutchins, C.W., Steffey, M.E., MacKenzie, R.G. and Frail, D.E. (1992) J. Biol. Chem. 267, 17780–17786.
[37] Strader, C.D., Dixon, R.A.F., Cheung, A.H., Candelore, M.R., Blake , A.D. and Sigal, I.S. (1987) J. Biol. Chem. 262, 16439–16443.
[38] Dalman, H.M. and Neubig, R.R. (1991) J. Biol. Chem. 266, 11025–11029.
[39] Luttrell, L.M., Ostrowski, J., Cotecchia, S., Kendall, H. and Lefkowitz, R.J. (1993) Science 259, 1453–1457.
[40] Hawes, B.E., Luttrell, L.M., Exum, S.T. and Lefkowitz, R.J. (1994) J. Biol. Chem. 269, 15776–15785.
[41] Okamoto, T. and Nishimoto, I. (1992) J. Biol. Chem. 267, 8342–8346.
[42] Namba, T., Sugimoto, Y., Negishi, M., Irie, A., Ushikubi, F., Kakizuka, A., Ito, S., Ichikawa, A. and Narumiya, A. (1993) Nature 365, 166–170.
[43] Sugamori, K.S., Sunahara, R.K., Guan, H.-C., Bulloch, A.G.M., Tensen, C.P., Seeman, P., Niznik, H.B. and Van Tol, H.H.M. (1993) Proc. Natl. Acad. Sci. USA 90, 11–15.
[44] Weiss, S., Goldberg, J.I., Lukowiak, K. and Drummond, G.I. (1985) J. Comp. Physiol. B 156, 57–65.
[45] Lazareno, S. and Birdsall, N.J.M. (1993) Trends Pharmacol. Sci. 14, 237–239.
[46] Andersen, P.H., Gingrich, J.A., Bates, M.D., Dearry, A., Falardeau, P., Senogles, S.E. and Caron, M.G. (1990) Trends Pharmacol. Sci. 11, 231–236.
[47] Lankford, K.L., Demello, F.G. and Klein, W.L. (1988) Proc. Natl. Acad. Sci. USA 85, 2839–2843.
[48] Rodrigues, P.D.S. and Dowling, J.E. (1990) Proc. Natl. Acad. Sci. USA 87, 9693–9697.
[49] Dowling, J.E. (1994) in: Dopamine Receptors and Transporters (Niznik, H.B. ed.) pp. 37–57, Marcel Dekker, New York.
[50] Davis, L. and Ish-Horowicz, D. (1991) Cell 67, 927–940.
[51] Xu, M., Moratalla, R., Gold, L.H., Hiroi, N., Kob, G.F., Graybiel, A.M. and Tonegawa, S. (1994) Cell 79, 729–742.

[52] Gotzes, F., Balfanz, S. and Baumann, A. (1994) Receptors Channels 2, 131–141.
[53] Zhou, Q. Y., Grandy, D.K., Thambi, L., Kushner, J.A., Van Tol, H.H.M., Cone, R., Pribnow, D., Salon, J., Bunzow, J.R. and Civelli, O. (1990) Nature 347, 76–80.
[54] Dearry, A.G., Gingrich, J., Falardeau, P., Fremeau, R.T., Bates, M.D. and Caron, M.G. (1990) Nature 347, 72–76.
[55] Machida, C.A., Searles, R.P., Nipper, V., Brown, J.A., Kozell, L.B. and Neve, K.A. (1992) Mol. Pharmacol. 41, 652–659.
[56] Nash, S.R., Godinot, N. and Caron, M.G. (1993) Mol. Pharmacol. 44, 918–925.
[57] Frail, D.E., Manelli, A.M., Witte, D.G., Lin, C.W., Steffey, M.E. and Mackenzie, R.G. (1993) Mol. Pharmacol. 44, 1113–1118.
[58] Weinshank, R.L., Adham, N., Macchi, M., Olsen, M.A Branchek, T.A. and Hartig, P.R. (1991) J. Biol. Chem. 266, 22427–22435.
[59] Grandy, D.K., Zhang, Y., Bouvier, C., Zhou, Q.-Y., Johnson, R.A., Allen, L., Buck, K., Bunzow, J.R., Salon, J. and Civelli, O. (1991) Proc. Natl. Acad. Sci. USA 88, 9175–9179.
[60] Tiberi, M., Jarvie, K.R., Silvia, C., Falardeau, P. Gingrich, J.A., Godinot, N., Bertrand, L., Yang-Feng, T.L., Fremeau, R.T.J. and Caron, M.G. (1991) Proc. Natl. Acad. Sci. USA 88, 7491–7495.