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Drosophila 5-HT_2 serotonin receptor: Coexpression with fushi-tarazu during segmentation

(*in situ* hybridization/G protein-coupled receptors/pair-rule gene)

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ABSTRACT **Serotonin, first described as a neurotransmitter in invertebrates, has been investigated mostly for its functions in the mature central nervous system of higher vertebrates. Serotonin receptor diversity has been described in the mammalian brain and in insects. We report the isolation of a cDNA coding for a *Drosophila melanogaster* serotonin receptor that displays a sequence, a gene organization, and pharmacological properties typical of the mammalian 5-HT_2 serotonin receptor subtype. Its mRNA can be detected in the adult fly; moreover, a high level of expression occurs at 3 hr of *Drosophila* embryogenesis. This early embryonic expression is surprisingly organized in a seven-stripe pattern that appears at the cellular blastoderm stage. In addition, this pattern is in phase with that of the even-parasegment-expressed pair-rule gene fushi-tarazu and is similarly modified by mutations affecting segmentation genes. Simultaneously with this pair-rule expression, the complete machinery of serotonin synthesis is present and leads to a peak of ligand concomitant with a peak of 5-HT_2-specific receptor sites in blastoderm embryos.**

In vertebrates, the biogenic amine serotonin (5-hydroxytryptamine, 5-HT) affects a wide variety of behavioral and physiological functions that are mediated by numerous receptor subtypes. These receptors can be classified according to the transduction mechanisms: the 5-HT_1 subtype as an adenylyl cyclase inhibitor, 5-HT_2 as a phospholipase C stimulator, and 5-HT_4, 5-HT_6, and 5-HT_7 as adenylyl cyclase activators; these are all G protein-coupled receptors. The 5-HT_3 subtype is a ligand-gated channel (1).

In insects, biogenic amines are similarly implicated as neuromodulators and neurotransmitters (2) in addition to their function of crosslinking proteins and chitin during sclerotization of the cuticle (3). In *Drosophila melanogaster*, the serotoninergic neurons have been localized (4) and implicated in salivary gland secretion, heart and oviduct contractions, circadian rhythms, and learning and memory (2). 5-HT receptors have also been detected as a heterogeneous population by [^{3}H]5-HT binding experiments or by second-messenger coupling to adenylyl cyclase or to phospholipase C (2). So far, only receptors coupled to adenylyl cyclase and close to the mammalian 5-HT_1 receptor subfamily have been described in *Drosophila* (5).

The pathway leading to 5-HT uses tryptophan as a substrate, which is converted to 5-hydroxytryptophan by tryptophan 5-hydroxylase (TPH; EC 1.14.16.4, the rate-limiting enzyme in 5-HT synthesis). 5-Hydroxytryptophan is converted to 5-HT by dopa decarboxylase (EC 4.1.1.28). Depletion of 5-HT and dopamine in *Drosophila* mutants lacking dopa decarboxylase leads to learning deficits, to an aberrant pattern of serotonergic neurons (6), and to incomplete sclerotization of the cuticule (3).

We report the characterization of a *Drosophila* serotonin receptor that displays a typical 5-HT_2 receptor sequence, gene organization, and pharmacology.¶ It is expressed in the central nervous system (CNS) during larval and adult stages. More surprisingly, this receptor is expressed at the blastoderm stage of embryogenesis in a pattern similar to that of the pair-rule gene fushi-tarazu (*ftz*).

MATERIALS AND METHODS

Fly Stocks. From the stocks described in ref. 7, we used the following, hypomorph or null, alleles: hairy (*h*), [25]5H07; runt (*run*), [28]YE96; even-skipped (*eve*), [3]R13; fushi-tarazu (*ftz*), [13]9H34; Krüppel (*Kr*), [2]; hunchback (*hb*), Df(3R)p25; knirps (*kni*), [11]IIE72; giant (*gt*), YA82; tailless (*tll*), [1]L10; engrailed (*en*), [54]IIB86; odd-skipped (*odd*), [5]IIID36; and snail (*sna*), IIG05.

Drugs and Chemicals. Ketanserin, ritanserin, and setoperone were kindly provided by Janssen, and ICS 205-930 and MDL 72 222 by Sandoz Pharmaceutical and Merell–Dow (Strasbourg, France), respectively. Other neurochemicals were from Research Biochemicals (Natick, MA) or Sigma. (±)-1-(2,5-Dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]-DOI) (2200 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear.

PCR Experiments. For receptor cloning, degenerate oligonucleotides coding for conserved sequences of transmembrane domains VI (a) and VII (b and c) were used for PCR amplification as described (8): a, 5′-TACCTCGAGGTCGACGGTI-ATGTGGTGYCCITTYTTYAT-3′; b, 5′-AGAACTAGTGG-TACCCRTIGTRTADATIAYIGGRTT-3′; and c, 5′-AGAAC-TAGTGGTACCCSWRCAIACRTAICCDATCCA-3′.

***In Situ* Hybridization.** Digoxigenin-labeled NP81 cDNA (see *Results*) was synthesized and was detected with alkaline phosphatase, using 5-bromo-4-chloro-3-indolyl phosphate as substrate, on whole-mount embryos (8). For double-staining experiments, after *in situ* hybridization, embryos were washed and incubated with a mouse anti-en monoclonal antibody (provided by M. Haenlin, Institut de Génétique et de Biologie Moléculaire et Cellulaire) or with a rabbit anti-ftz antiserum (1:1000; provided by W. J. Gehring, University of Basel, Basel). The signal was detected by a peroxidase-coupled rabbit anti-mouse or donkey anti-rabbit secondary antibody (1:350; Cappel) with diaminobenzidine as substrate.

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Abbreviations: AEL, after egg laying; CNS, central nervous system; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 5-HT, 5-hydroxytryptamine (serotonin); TPH, tryptophan 5-hydroxylase.

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X81835 and X85407 for the cDNA and genomic sequences, respectively).

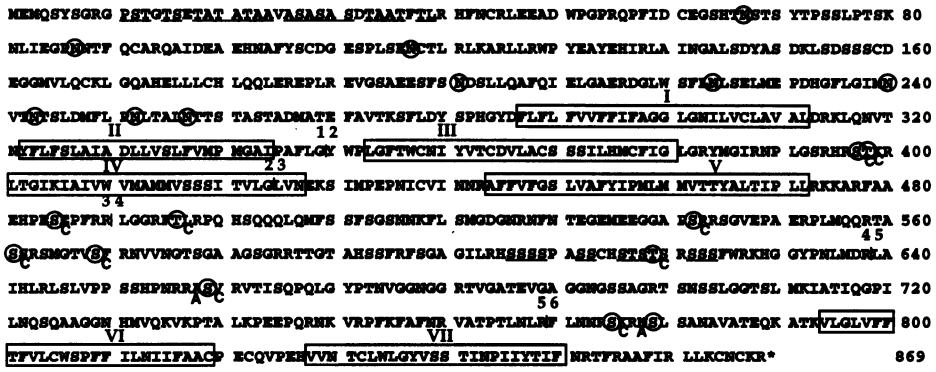

FIG. 1. Deduced amino acid sequence of the NP81 receptor. Roman numerals above boxes identify the transmembrane domains. The intron/exon boundary locations are indicated by the exon numbers (Arabic numerals above the sequence). Putative N-glycosylation sites are circled and the consensus phosphorylation sites by protein kinase A or C are circled and labeled with left inferior "A" or right inferior "C," respectively. The hydrophobic region near the N terminus and the serine/threonine-rich region in the third intracellular loop are underlined.

Binding Assay. [^{125}I]DOI (9) was used as the radioligand on membrane fractions isolated from embryos or from NP81-transfected COS-1 cells as described (10).

5-HT Dosage. Extracts from staged embryos were used for binding experiments or for 5-HT quantification by enzymatic–isotopic microassay using partially purified *N*-acetyltransferase (11) or for determination of TPH activity by a radioenzymatic assay (12). The resulting global concentration was deduced from the calculated maximum number of binding sites (B_{max}) by assuming the *Drosophila* embryo volume as 15 nl and a uniform distribution over the embryo.

RESULTS

NP81 cDNA Encodes a 5-HT$_2$-Like Receptor. By PCR amplification, we cloned a *Drosophila* genomic DNA fragment, NP81, that we used to screen a library of *Drosophila* head cDNA. The complete cDNA sequence (3889 bp) encodes a protein of 868 aa (Fig. 1) which displays eight hydrophobic regions, seven of which can be identified as transmembrane domains (Fig. 1). The NP81 protein shares homologies with the G protein-coupled receptor family, including (*i*) potential N-glycosylation sites in its amino terminus, (*ii*) consensus sequences for phosphorylation by different protein kinases in the cytoplasmic regions, and (*iii*) an identical location for conserved amino acids within the transmembrane regions (13) (Fig. 1). The genomic sequence of the NP81 gene, investigated by screening a *Drosophila* genomic library in λ phage EMBL4, spans 10 kb and consists of six exons interspersed with five introns.

In situ hybridization on salivary gland polytene chromosomes (data not shown) located the NP81 receptor gene on the right arm of the third chromosome in the region 82C–82F uncovered by the deficiency Df(3R)110, which contains the pair-rule gene odd-paired (*opa*) located in 82E (14). However, the NP81 gene is still present in embryos homozygous for the

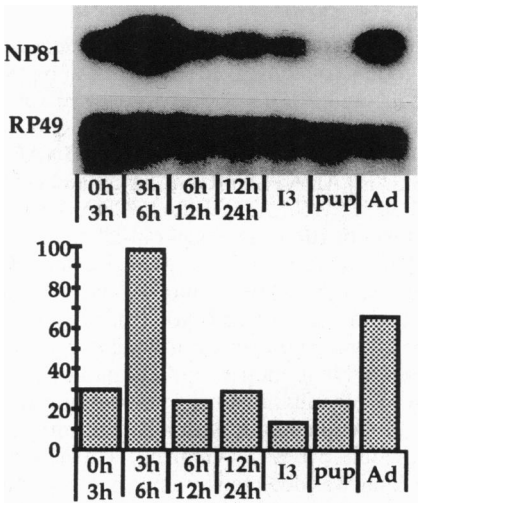

FIG. 2. Developmental regulation of NP81 mRNA. Total RNA from various stages of development was used for reverse transcription–PCR with NP81- and ribosomal protein 49 (RP49)-specific primers. PCR products were revealed by hybridization to ^{32}P-labeled NP81 or RP49 probes. The histogram shows the relative amount of mRNA after scanning of the autoradiogram and normalization to the RP49 signal. Arbitrary units of the scale on the right are relative to the expression (100) seen in the embryos at 3–6 hr after egg laying (AEL). I3, third-instar larval RNA; pup, pupal RNA; Ad, adult RNA.

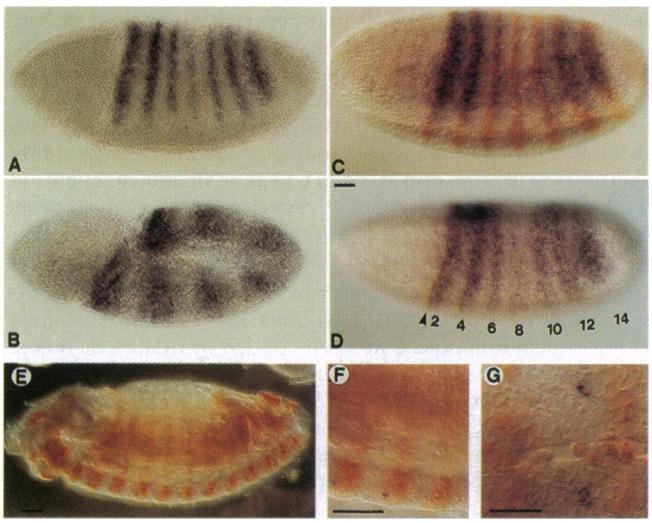

FIG. 3. NP81 mRNA is expressed in seven stripes. Wild-type embryos were probed by *in situ* hybridization with digoxigenin-labeled NP81 cDNA. Embryos are shown anterior to the left, ventral side down. (*A–D*) Reaction products are seen as blue/violet staining for the NP81 mRNA. (*A*) *Drosophila* embryo corresponding to the cellular blastoderm stage (stage 6). The NP81 mRNA is expressed as seven stripes, each about four cells wide. The pattern differs from the typical pair-rule striping in two ways: (*i*) the intensity of the different stripes is variable (2 > 1 > 7 > 6 > 3 = 5 > 4) and (*ii*) the ventral part of the embryo corresponding to the presumptive mesoderm is not labeled. (*B*) *Drosophila* embryo at 4 hr, corresponding to germ-band extension (stage 9): the staining remains in seven stripes. (*C* and *D*) Double labeling of similarly-treated stage 6 embryos further processed by incubation with anti-ftz polyclonal (*C*) or anti-en monoclonal (*D*) antibody and revealed by peroxidase: the labeling is seen as a yellow-brown color. (*C*) The NP81-positive stripes are localized in the same parasegments as ftz. (*D*) The first en-positive cells colocalize with the anterior edge of the first NP81-positive stripe. Numbers correspond to parasegments and the arrowhead indicates the second en stripe. (*E*) Lateral view of a stage 13 embryo showing NP81 *in situ* hybridization in the CNS and stained with antibody to en. (*F*) Higher magnification of *E* showing one NP81-positive cell per neuromere in front of the en stripe. (*G*) High magnification of a ventral view of a stage 13 embryo, showing the two NP81-positive cells on both sides of the ventral midline and in front of the en stripe. (Bars = 25 μm except in *G*, 12.5 μm.)

deficiency Df(3R)6-7, which uncovers the region $82D_3$–D_8; 82F.

NP81 Protein Displays a 5-HT_2 Pharmacology. ^{125}I-DOI, a 5-HT_2-specific radioligand, interacted with membranes prepared from NP81-transfected COS-1 cells (and not with membranes from mock-transfected cells) with high affinity and in a saturable fashion (k_{+1} = 0.123 × 10^7 M^{-1}·min^{-1}, k_{-1} = 0.0166 min^{-1}). The saturation data exhibit a best-fit to a single-site mode with apparent B_{max} of 8.7 pmol/mg of protein and K_d of 12.8 ± 5.5 nM (n = 3). Competitive inhibition studies gave the following rank order of potencies for selected drugs: ritanserin > ketanserin > pizotifen > 5-HT > setoperone > spiperone = *N*-(3-trifluoromethylphenyl)piperazine = cyproheptadine = mesulergine = *N*-acetyl-5-HT > methiothepine = methysergide (see Table 1). LY 53,857 was noncompetitive, and the binding of chlorpromazine and of the other amines histamine, dopamine, tyramine, and octopamine was not significant ($pK_i \leq 4$).

Expression of NP81 mRNA. Quantitative reverse transcription–PCR analysis demonstrated that the NP81 mRNA was expressed at a high level after 3 hr of embryonic development and that after a downregulation it reappeared later in embryogenesis, in the larval stage, and in the adult (Fig. 2). In agreement, the NP81 mRNA was first detected by *in situ* hybridization on whole-mount embryos at the beginning of cellular blastoderm (stage 6; 2 hr 50 min AEL) (15). This detection revealed seven evenly spaced transverse stripes along the anteroposterior axis of the embryo, a pattern similar to that of the pair-rule genes (Fig. 3 *A–D*) (14). Syncytial expression was not detectable, but the striped pattern appeared before cephalic furrow formation, at the onset of cellularization. It was restricted to the ectodermal layer of the blastoderm embryo in four-cell-wide stripes with uneven intensity among each parasegment (Fig. 3*A*). The seven stripes persisted during germ-band extension (Fig. 3*B*) and then the expression disappeared. The NP81 mRNA stripes were in phase with those of cells expressing the pair-rule gene *ftz* (Fig. 3*C*). The colocalization of the earliest-appearing stripe of *en*-expressing cells with the anterior margin of the most anterior NP81 stripe (Fig. 3*D*) located the second parasegment (16) and therefore confirmed the NP81 phasing with *ftz* in the even-numbered parasegments. NP81 mRNA reappeared in the embryo ventral nerve cord as well as in the larval CNS in a pair of cells per neuromere, starting at stage 13 (Fig. 3 *E–G*).

Pattern of NP81 mRNA Expression in a Mutant Background. The blastoderm expression of the NP81 mRNA in gap-gene mutant (*Kr, hb, kni, gt*, and *tll*) embryos was affected in a similar way as that of *ftz*, the other even-parasegment-specific gene (Fig. 4 *F–J*) (17, 18). Mutations in primary pair-rule genes (*h, run*, and *eve*) globally induced similar

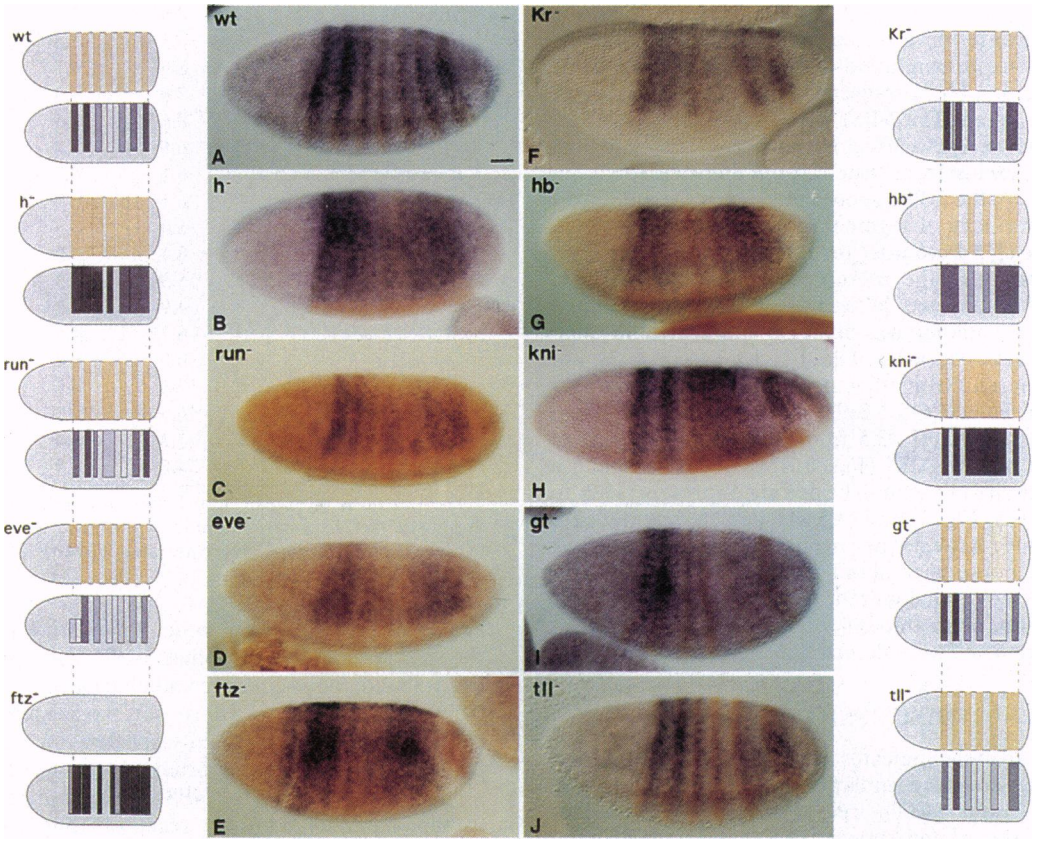

FIG. 4. NP81 expression in a mutant background. Homozygous mutant embryos at the cellular blastoderm stage (stage 6) were double labeled with the NP81 probe and the anti-ftz antibody as in Fig. 3. At right and left are schematic interpretations of the observed patterns, ftz in yellow on top and NP81 in blue. (*A*) Wild-type (wt) pattern. (*B–E*) Pair-rule mutants. (*B*) In *h* mutant, the NP81 periodicity is nearly lost, like ftz, except in the middle of the embryo on both sides of the fourth stripe. (*C*) In *run* mutant the overall pattern is weaker than the wild type, as for ftz. The relative intensity of the three middle stripes is changed (3 > 4 > 5). (*D*) In *eve* mutant, the stripes are shifted toward the anterior part and weaker than the wild type, as for ftz. The first stripe has lost its posterior limit and, in contrast to ftz, is ventrally restricted. (*E*) In *ftz* mutant, NP81 staining is still visible although its restriction to even-numbered stripes is not anymore obvious. The coloration has been overstained, and this mutated pattern has been confirmed by double staining with anti-en antibody showing a lack of even stripes. A similar pattern is seen with the ftz^{w20} null allele (data not shown). (*F–J*) Gap-gene mutants. (*F*) In *Kr* mutant the NP81 mRNA is restricted to five stripes with a large gap between the third and the fourth stripe and with the second stripe proportionally stronger than ftz. (*G*) In *hb*-deficient background there is a complete fusion of the two most anterior stripes and of the most posterior stripes. (*H*) In *kni* mutant, the NP81 pattern presents a wide band that extends across the area where the third through sixth stripes normally form. (*I*) In gt mutant, the two most anterior stripes are strong and the fifth and sixth stripes are mixed and weaker than in the wild type. (*J*) The *tll* mutant presents only six stripes expanded over the normal trunk region, like ftz. (Bar = 25 μm.)

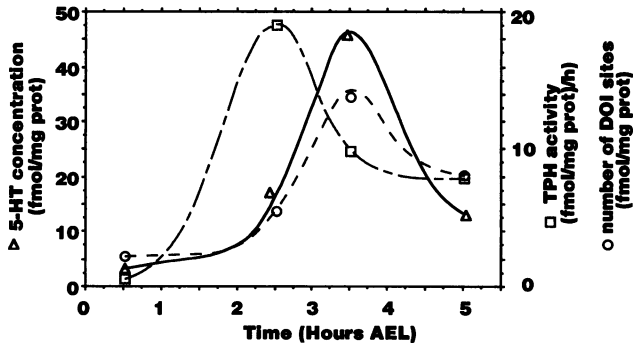

FIG. 5. 5-HT biochemistry of early *Drosophila* embryos. Extracts from *Drosophila* embryos collected at early periods of embryogenesis (0–1 hr, 2–3 hr, 3–4 hr, and 4–6 hr AEL) were used to determine the presence of 5-HT_2-like receptor binding sites by ^{125}I[DOI] binding. TPH activity and 5-HT were determined by radioenzymatic assay. Values for 0–1 hr AEL are all below the detection limits of the assays: <2 fmol/mg of protein for DOI B_{max}, <0.3 fmol/hr per mg of protein for TPH activity, and <1.5 fmol/mg of protein for 5-HT. The curves are representative of at least three experiments performed in triplicate.

modifications in the NP81 and *ftz* patterns (Fig. 4 *B–D*) (17). Finally, the NP81 pattern in *ftz* mutants displayed important modifications: a partial loss of stripe restriction, shift of the first stripe to the anterior of the cephalic furrow, and enlargement of the low-expressing middle region to the third stripe (Fig. 4*E*). Therefore, NP81 transcription is not strictly dependent on *ftz* expression. The NP81 pattern was unaffected for mutated loci known not to affect *ftz*, such as *en* and *odd*, and the mesodermic exclusion persisted in *sna* embryos (data not shown). Therefore, the NP81 gene is located in the vicinity of *ftz* within the hierarchy of segmentation genes.

Biochemistry of Serotoninergic Molecules in Early Embryos. We investigated the presence of receptor protein in *Drosophila* embryo extracts by analyzing the binding of the [^{125}I]DOI. Specific binding was detected and Scatchard analysis of the data revealed a K_d of 15.4 ± 1.6 nM (n = 3), not significantly different from that determined in membranes from NP81-transfected COS-1 cells (K_d of 12.8 ± 5.5 nM) and the presence of a peak of 13.5 fmol of receptor per mg of embryo protein 3–4 hr AEL (Fig. 5). Similarly, we found a peak of TPH activity (18.8 fmol/hr per mg of protein) in 2- to 3-hr-AEL embryos (Fig. 5) and of 5-HT detected by radioenzymatic assay (11) (46.5 fmol per mg of protein corresponding to a global concentration of about 15 nM) in 3- to 4-hr-AEL embryos. Therefore, concomitant with the peak of NP81 mRNA expression, peak amounts of specific 5-HT_2 receptor binding sites and ligand were detected in blastoderm embryos.

DISCUSSION

The sequence analysis indicates that the NP81 gene and protein structures are more similar to those of the mammalian 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors than to any other *Drosophila* receptor so far known. The original long amino terminus and the long third intracellular loop containing serine/threonine repeats (Fig. 1) are also present in the *Drosophila* muscarinic acetylcholine receptor and 5-$HT1_{Dro2A}$ receptors.

A comparison of the NP81 pharmacological properties (Table 1) with those of the rat cortex 5-HT_{2A}, COS-1-expressed mouse 5-HT_{2B}, or pig choroid plexus 5-HT_{2C} receptors (10, 19), using the nonparametric Spearman rank correlation test, shows that the best correlation is seen with the mouse 5-HT_{2B} receptor antagonists (r_S = 0.864, P < 0.001) and for the 5-HT_{2B} agonists (r_S = 0.653, P < 0.05) and that no significant correlation is seen for the 5-HT_{2A} and 5-HT_{2C} agonists (Table 2) or any other serotonin receptor (data not shown). Although Drosophila 5-HT_1 subtypes have already been cloned, their sequence and pharmacology appear distinct from those of NP81 (5). Moreover, the existence of 5-HT_2-like receptors in insects has been suspected since inositol trisphosphate stimulation was reported in response to 5-HT in *Drosophila* (2). Therefore, the NP81 receptor appears to be similar to the mammalian 5-HT_2 receptors but distinct from the

Table 1. Competition with [^{125}I]DOI for membranes from NP81 cDNA-transfected COS-1 cells as compared with published values for mammalian 5-HT_2 receptors

Drug	pKi			
	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	NP81
Agonists				
N-Acetyl-5-HT	5.5	8.0	6.1	7.2
Tryptamine	6.0	6.7	7.2	5.8
5-CT	3.5	6.5	5.7	4.4
(±)α-Methyl-5-HT	7.3	6.9	7.3	6.8
5-HT	5.5	5.9	7.5	7.8
1-Methyl-5-HT	6.3	5.6	8.4	5.5
5-Methoxytryptamine	5.5	5.5	7.6	5.3
8-OH-DPAT	5.0	5.2	5.2	4.9
Quipazine	6.2	5.2	6.7	5.2
2-Methyl-5-HT	5.2	5.2	5.8	5.8
5-Methyltryptamine	5.2	5.0	8.1	4.9
N,N-Dimethyl-5-methoxy-tryptamine	6.2	5.1	7.0	4.2
RU 24969	6.0	4.8	6.5	4.3
Antagonists				
Ritanserin	9.3	8.4	8.6	8.3
Pizotifen	7.8	8.2	8.1	8.1
Methysergide	8.6	7.9	8.6	7.1
Mesulergine	8.4	7.7	8.8	7.2
Methiothepine	8.8	7.5	7.6	7.1
Cyproheptadine	8.5	7.6	7.9	7.2
Ketanserin	8.9	6.7	7.0	8.2
Spiperone	8.8	7.3	5.9	7.2
Setoperone	8.6	7.6	7.3	7.7
TFMPP	6.6	7.2	7.2	7.2
Rauwolscine	6.1	6.7	5.8	6.7
Buspirone	6.1	6.5	5.1	6.5
Yohimbine	6.0	6.3	4.4	6.4
Bufotenine	6.4	5.9	7.2	6.0
ICS 205-930	5.3	5.3	4.6	4.9
Iodocyanopindolol	4.9	4.8	5.0	4.8
MDL 72 222	6.7	4.6	5.0	4.7
Mianserin	8.0	6.4	8.1	5.7
cis-Flupenthixol	7.6	6.6	6.1	5.4
Haloperidol	6.6	6.5	4.8	5.3
Clozapine	7.2	6.4	5.9	5.5
Sulpiride	4.5	4.9	4.1	6.0

Each value is the mean of at least three independent trials run in duplicate. 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-(di-*n*-dipropylamino)tetralin; TFMPP, *N*-(3-trifluoromethylphenyl)piperazine.

Table 2. Spearman rank correlations calculated from the comparison of the pKi values of the NP81 receptor with those of the mammalian 5-HT_2 receptors

Receptor subtype	Agonists plus antagonists (n = 35)		Antagonists (n = 22)		Agonists (n = 13)	
	r_S	P	r_S	P	r_S	P
5-HT_{2A}	0.584	<0.001	0.699	<0.001	0.293	NS
5-HT_{2B}	0.797	<0.001	0.864	<0.001	0.653	<0.05
5-HT_{2C}	0.437	<0.01	0.606	<0.005	0.217	NS

Drosophila 5-HT_{1Dro}, 5-HT_{Dro2A}, and 5-HT_{Dro2B} receptors. In accordance with the new mammalian nomenclature (19), we propose that NP81 be called 5-HT_{2Dro} and that 5-HT_{Dro2A}, 5-HT_{Dro2B}, and 5-HT_{1Dro} be renamed 5-HT_{1ADro}, 5-HT_{1BDro}, and 5-HT_{7Dro}, respectively.

Blastoderm 5-HT_{2Dro} expression is highly regulated. (*i*) The receptor mRNA was detected by PCR amplification and *in situ* hybridization, and a concomitant peak of DOI binding was detected in blastoderm embryo (Figs. 2, 3, and 5). (*ii*) This pattern is different from that of any known pair-rule gene (Fig. 3); it coincides with but does not exactly match the *ftz* pattern. (*iii*) The *in situ* hybridization signal detected during late embryogenesis in two cells per neuromere (Fig. 3 *E–G*) is distinct from the *ftz*-positive CNS cells and seems to be different from that of other published 5-HT receptors (5); the CNS pattern is compatible with that of 5-HT-positive neurons (4), but the position of the 5-HT_{2Dro}-positive cells in front of the *en*-positive stripe (Fig. 3 *E–G*) makes it highly unlikely that they correspond to the progeny of neuroblast 7-3, shown in grasshopper to give rise to 5-HT neurons (20) and to be *en*-positive (21). (*iv*) 5-HT_{2Dro} expression is modified in gap and pair-rule mutant backgrounds, mostly in a similar way to *ftz*, which is expected for a colocalized gene product (Fig. 4). However, the restriction to the ectodermal part of the embryo persists, as does the gradient of expression toward the middle of the embryo. In addition, differences are noticeable in the *Kr, eve, run*, and *h* backgrounds and the pattern is modified but persists in the *ftz* mutant. However, if *ftz* is dispensable for initiation of the 5-HT_{2Dro} striped expression, it could be necessary to refine or maintain this pair-rule expression. Therefore, the 5-HT_{2Dro} striped pattern is not directly dependent upon any one of the tested segmentation genes and seems to result from a combination of the gap and pair-rule genes affecting *ftz*, on one hand, and *ftz* itself, on the other.

The detection in blastoderm embryos of DOI binding sites with affinity identical to that of the 5-HT_{2Dro} receptor (Fig. 5) demonstrates that 5-HT_2-like receptors are present. A peak of TPH activity (Fig. 5) correlated with the presence of its mRNA (22) and a peak of dopa decarboxylase activity described at the same embryonic stage (23) indicate that the ligand, 5-HT, is synthesized zygotically (Fig. 5). Therefore, accompanying the transient 5-HT_{2Dro} mRNA expression, receptor and ligand are synthesized in the blastoderm embryos.

Since the known pair-rule genes encode transcription factors, with the exception of the recently characterized gene *oddOz* (24), also called *ten^m* (25), and since 5-HT_{2Dro} expression starts at the onset of embryonic cellularization, when the patterning process needs to integrate a new morphological organization, the 5-HT_{2Dro} receptor gene may represent another type of *Drosophila* patterning gene. Signal transduction by the 5-HT_2 receptors is known to stimulate the protein kinase C, which may phosphorylate transcription factors, among other substrates. Interestingly, the ftz protein, a homeodomain-containing transcription factor, is specifically phosphorylated on serine and threonine residues at 3–4 hr AEL (26). Therefore, such a receptor molecule could transduce an unrestricted signal into a segmental modulation of preexisting molecules.

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