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Early expression of a β_1-adrenergic receptor and catecholamines in *Xenopus* oocytes and embryos

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Abstract From a *Xenopus* stage 11 cDNA library, we have cloned a gene, termed *X-βIAR*, whose sequence is highly homologous to that of the human β_1-adrenergic receptor. As shown by RT-PCR assay, *X-βIAR* RNA is present in the mature oocyte, decreases after fertilization up to stage 6 and then gradually increases during gastrulation. Binding studies performed with radiolabeled ligands reveal that *X-βIAR* RNA is translated into the receptor protein. Furthermore, noradrenaline and adrenaline are also detected in oocytes and early embryos. The concomitant presence of β_1-adrenergic receptors and catecholamines suggest that this ligand-receptor couple could play a role in the very early stages of embryonic development.

Key words: Early embryogenesis; *Xenopus*; β-Adrenergic receptor; Catecholamine

1. Introduction

Early embryogenesis is associated with key development events, such as determination of various cell lineages and specification of body axes. The generation of diversity and pattern are interdependent in animal development and contribute to the patterning of the whole embryo.

Before fertilization, the *Xenopus* oocyte already comprises the prospective ectoderm in the pigmented animal pole and the prospective endoderm in the yolky vegetal pole. The third embryonic layer, mesoderm, is generated during the segmentation following fertilization and proceeds through the emission of an inductive signal from the endoderm to the overlying ectoderm [1]. Mesoderm induction can begin as early as the 32-cell stage [2] and mesodermal cells are already patterned along the dorso-ventral axis. Indeed, the specification map of early mesoderm shows a localized region of dorsal character, fated to form notochord and head mesoderm, and an extended ring, of ventral character, fated to form muscle, lateral plate mesoderm and blood [3].

These patterning and inductive events are accomplished through cell interactions which involve the emission and the decoding of various signals. Over the past years, diffusible signals have been shown to be expressed during early embryogenesis and to display biological activity with regard to the patterning and induction of mesoderm.

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The active molecules in mesoderm induction are peptide growth factors belonging to two families, the TGF-β family with activins [4,5], bone morphogenetic protein 4 (BMP-4) [6] and Vg1 [7], and the FGF family [8,9]. The inducing properties of these two families of molecules are not identical, as the FGF isoforms only induce ventrolateral mesoderm [10] whereas activins and Vg1 can induce all types of mesoderm [7,11]. Interestingly, activins display a concentration-dependent activity with respect to the induction of mesoderm subtypes [12], suggesting that a gradient of this molecule along the dorso-ventral axis could be the molecular basis for mesoderm patterning.

The receptors which bind the ligands belonging to the FGFs [13,14] or to the TGF-β family [15,16] were later demonstrated to be expressed during early embryogenesis. All these receptors belong to the class of receptor enzymes which contain a kinase activity on the same polypeptide chain as the ligand-binding site.

We wished to ascertain whether other classes of receptors involved in signal transduction were likely to participate in early embryogenesis. As transcripts encoding the α and β subunits of GTG-binding proteins are expressed at early embryonic stages [17–20], we looked for the concomitant expression of G protein-coupled receptors. Using primers located in consensus sequences of transmembrane segments of G protein-coupled receptors as described [21], we amplified two major cDNA fragments in a reaction mixture containing reverse-transcribed mRNA purified from *Xenopus* gastrulas. The smallest amplified fragment allowed us to isolate a full-length cDNA which encodes a new G protein-coupled receptor, X-msr, whose expression is associated with an endothelial lineage [22].

We report here on the characterization of the largest amplified fragment, the cloning of the corresponding cDNA and its expression during early embryogenesis.

2. Materials and methods

2.1. Embryos

Fertilized embryos were obtained as previously described [23]. Staging was established according to the tables of Nieuwkoop and Faber [24].

2.2. RNA extraction

Total RNA was extracted from 10 staged *Xenopus* embryos by the addition of 900 μl RNA-B (Bioprobe). Phenol extraction was performed after addition of 100 μl chloroform. RNA was isolated by isopropanol precipitation of the aqueous layer and then dissolved in DEPC-treated water.

2.3. PCR amplification of G protein-coupled receptor sequences

In an effort to clone *Xenopus* serpentine receptor genes by PCR

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amplification, we used degenerate oligonucleotides located in sequences of transmembrane domains III and VI, as defined [21]: primer III-5′, 5′-AAAGGATCCCTGTG(C/T)G(C/T)(G/C)AT(C/T)GCNNT(G/T)GA(C/T)(C/A)G(C/G)T AC-3′; primer VI-3′, 5′-AAGAATTCA(T/G)G(A/T)AG(A/T)AGGGCAGCCAGCAGAN(G/C)(G/A)(T/C)GA-A-3′. cDNA was synthesized in a 20 μl reaction mixture containing 5 μg of total RNA isolated from staged 10 embryos using the Super-Script II Gibco BRL kit. 1 μl cDNA was used as a template in a 50 μl amplification mixture containing 200 μM dNTPs, 2.5 U Taq DNA polymerase (Boehringer Mannheim) and 0.5 μM of each primer. During the 35 cycles of the reaction, the denaturing, annealing and extension steps were optimized for 1 min at 94°C, 55°C, 72°C, respectively. The 500 bp amplified fragment was purified by the GeneClean procedure (Bio-101) and subcloned in a pIBI vector. The insert was sequenced using a Sequenase II kit (USB).

2.4. Cloning of the full-length serpentine receptor cDNA

The *Xenopus laevis* cDNA library was made at developmental stage 11 in λgt 10 by Krieg and Melton [25]. This library was plated on *Escherichia coli* C600/hfla$^-$ at a density of 20000–30000 plaques per 140 mm plate. Recombinants were transferred to nitrocellulose filters (Hybond C, Amersham), denatured, baked for 2 h in vacuo at 80°C and prehybridized in 50% formamide, 5×SSC, 2×Denhardt's solution, 0.1% SDS at 42°C for 3 h. Hybridization was carried out for 16 h at 42°C, in the same buffer containing 50 μg/ml sonicated salmon sperm DNA with, as probe, 2×10^6 cpm per filter of the 500 bp amplified cDNA fragment corresponding to the coding region between the third and the sixth transmembrane segments, randomly labeled with [^{32}P]α-dCTP (Amersham). The filters were washed in buffers of decreasing stringency down to 0.1×SSC, 0.1% SDS at 42°C and autoradiographed overnight. Positive clones were purified and subjected to secondary and tertiary screening. The selected clones were digested with *Eco*RI and subcloned in pIBI31 vector. The insert was then sequenced with a Sequenase II kit (USB).

The 5′ region was isolated by a PCR-based strategy using the 5′ RACE method. Amplification of the 5′ coding region was performed using standard procedures of the 5′ RACE-amplifinder kit (Clontech): RNA from stage 10 embryos was reverse-transcribed with a specific P1 primer corresponding to nucleotides 729–752. The PCR amplification was then carried out with a primer containing an anchor and a specific P2 primer corresponding to nucleotides 275–294. An amplified 599 bp fragment was identified and found to overlap the nucleotide sequence of the inserts. The sequence given in Fig. 1 is the cumulative full-length cDNA resulting from the assembly of the overlapping regions obtained from the 5′ RACE-amplified fragment and the largest insert which includes most of the coding sequence (175–1155) and the 3′ untranslated region.

2.5. RT-PCR analysis of X-β1AR mRNA levels

The RNA extraction and reverse transcription were performed from 10 oocytes or staged embryos as described above. 4 μl cDNA was used as a template in a 50 μl amplification mixture containing 200 μM dNTPs, 2.5 U Taq DNA polymerase (Boehringer Mannheim) and 0.2 μM of each primer. The X-β1AR amplified fragment was obtained with a 5′ primer (274–292) and a 3′ primer (1257–1233) during 40 cycles of denaturing, annealing and extension steps optimized for 1 min at 94°C, 60°C, 72°C, respectively. The X-Gβ1 amplified fragment was obtained with a 5′ primer (183–204) and a 3′ primer (1021–1001) during 30 cycles of denaturing, annealing and extension steps optimized for 1 min at 94°C, 55°C, 72°C, respectively. 10 μl of PCR reaction was loaded on a 1% TAE agarose gel. After migration of the samples, the gel was dried at 70°C for 30 min, denatured for 20 min in 0.5 M NaOH/0.15 M NaCl and neutralized for 20 min in 0.5 M Tris pH 8/0.15 M NaCl. The same purified amplified fragments were randomly labeled with [^{32}P]α-dCTP and used as probes for hybridization.

2.6. Preparation of oocyte and embryo membranes

One hundred oocytes or staged embryos were homogenized in 1.5 ml homogenization buffer (25 mM HEPES, pH 7.8, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 30 μg/ml aprotinin) The homogenates were centrifuged at 10000×*g* for 5 min. The clear supernatants were then centrifuged at 47000×*g* for 30 min and the final pellet resuspended in 500 μl of binding buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM $MgCl_2$, 1 mM EDTA, 1 mM PMSF, 30 μg/ml aprotinin).

2.7. Binding studies with radioligands

30 μl of membranes was incubated in the presence of 10 μl effector and 10 μl radioligand. The incubation in the presence of 5 nM [^{3}H]CGP 20714A (46 Ci/mmol) was performed at room temperature for 30 min whereas that in the presence of 150 pM [^{125}I]CYP (2000 Ci/mmol) was carried out at 30°C for 90 min. The reaction was stopped by adding 3 ml of cold 50 mM Tris-HCl, pH 8 and filtered on Whatman GF/C filters. The filters were washed four times with 3 ml of the same buffer and then dried before counting.

2.8. Catecholamine dosage

Ten oocytes or staged embryos were homogenized in 300 μl 0.1 N $HClO_4$ containing 1 mM EDTA and 4 mM sodium metabisulfite. After centrifugation at 12000×*g* for 10 min at 4°C, the clear supernatant was further purified by classical absorption on alumina and with dihydroxybenzylamine as internal standard. Levels of noradrenaline and adrenaline were measured with high pressure liquid chromatography (HPLC) with electrochemical detection. The HPLC equipment consisted of a Model P1500 pump (Spectra system LC) and AS 3500 autosampler (Thermo Separation Products) and Coulochem II electrochemical detector 5200A (ESA Inc.). The analysis was made on a Hypersil ODS C18 column (5 μm, 150×4.6 mm i.d., Shandon HPLC) maintained at 31°C. The mobile phase was 0.27 mM citric acid hydrate, 50 mM anhydrous sodium acetate, 0.83 mM octanesulfonic acid, 1.07 mM EDTA and 8% methanol adjusted to pH 4.3. The flow rate of the solvent was 1 ml/min.

3. Results

3.1. Cloning of X-β1AR cDNA

During the search for serpentine receptors expressed during early embryogenesis of *Xenopus*, we cloned a cDNA encoding the amphibian homologue of a β-adrenergic receptor. The cloning was performed by PCR technology in which primers corresponding to transmembrane domains III and VI were used to amplified total *Xenopus* stage 10 cDNA, as described [21]. Two major PCR products, a 500 bp and a 432 bp fragment, were obtained and subcloned into the pIBI vector. The characterization of the 432 bp fragment and its related full-length cDNA has been published elsewhere [22]. DNA sequencing of the larger PCR product revealed that its deduced protein sequence contained two hydrophobic domains corresponding to potential transmembrane segments IV and V and several invariant residues of this receptor superfamily [26].

Four partial full-length cDNA clones were first isolated from a *Xenopus* stage 11 cDNA library [25] using the cloned 500 bp PCR fragment as a hybridization probe. Unfortunately, the largest insert recovered from these positive colonies appeared to lack 174 nucleotides in the 5′ coding region. We decided therefore to use the RACE methodology in order to obtain the full-length cDNA.

The overlapping sequences of RACE products and partial full-length cDNA provides an open reading frame (1155 bp) which encodes a protein of 385 amino acids with a predicted molecular mass of 45 kDa (Fig. 1). An ATG codon at residue +1 is in the context proposed for an initiation codon [27]. The hydropathicity plot of the amino acid sequence of X-β1AR reveals the presence of seven hydrophobic regions corresponding to the putative transmembrane domains. Furthermore, the protein sequence contains the invariant residues found in most serpentine receptors, regardless of their ligand, such as an aspartate residue in the second transmembrane domain, the DRY sequence at the end of the third transmembrane domain and a tryptophan residue in the middle of the fourth and sixth transmembrane segments [26].

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-300   ACC ACC AAC GTG GCA CCT GCA GCT GAG GGA CTA GAA AAC TCC ATA GCC CAG AGG AGT CAC
-240   TGG CAG CCA CAA CTG TAC TGA AAG TGT AGC AGC TCA CAA GCC CCC GGC TTA TTC ATC CAG
-180   GAG ACA GAG AGA CTG GCA CAG TCC AGC CCA GTG GCA CGA GAG TCT GCA CCA ACC AGG GGG
-120   AGT TAT AGT TTC TGG ACA CAA GAG ACT ACC TGG CAT CCC GCT GGC ACC GAC ACT TTC TTT
-60    CTG TTC TGA TAT TCT GTT GCC CAA ATG ATC TGA GGC TCC AGG CTA GGA CCT ATG CCC ATC

1      ATG GGA GAC GGT TGG GGG CCT ATG GAG TGC AGG AAC AGG TCT GGT ACC CCT ACA ACA GTG
1      M   G   D   G   W   G   P   M   E   C   R   N   R   S   G   T   P   T   T   V

61     CCC AGC CCT ATG CAC CCC CTG CCC GAG CTC ACT CAC CAG TGG ACT ATG GGA ATG ACT ATG
21     P   S   P   M   H   P   L   P   E   L   T   H   Q   W   T   M   G   M   T   M

121    TTC ATG GCG GCC ATC ATC CTC CTC ATC GTC ATG GGC AAC ATC ATG GTC ATT GTG GCC ATT
41     F   M   A   A   I   I   L   L   I   V   M   G   N   I   M   V   I   V   A   I

181    GGG AGG AAC CAG AGG CTC CAG ACC TTG ACC AAC GTC TTC ATC ACG TCC TTG GCT TGT GCC
61     G   R   N   Q   R   L   Q   T   L   T   N   V   F   I   T   S   L   A   C   A

241    GAC CTC ATT ATG GGT TTG TTT GTT GTG CCC CTT GGT GCC ACG TTG GTG GTG AGT GGC AGG
81     D   L   I   M   G   L   F   V   V   P   L   G   A   T   L   V   V   S   G   R

301    TGG CTG TAC GGG TCG ATA TTC TGT GAG TTC TGG ACG TCA GTG GAC GTA TTG TGC GTC ACG
101    W   L   Y   G   S   I   F   C   E   F   W   T   S   V   D   V   L   C   V   T

361    GCG AGT ATA GAG ACC CTG TGC GTC ATC TCC ATC GAC CGC TAC ATC GCC ATC ACC TCA CCC
121    A   S   I   E   T   L   C   V   I   S   I   D   R   Y   I   A   I   T   S   P

421    TTC CGC TAC CAG AGT CTC CTG ACC AAG GGC CGT GCC AAG GGA ATC GTG TGC AGC GTG TGG
141    F   R   Y   Q   S   L   L   T   K   G   R   A   K   G   I   V   C   S   V   W

481    GGC ATC TCA GCC CTG GTC TCG TTC CTG CCC ATC ATG ATG CAC TGG TGG AGG GAC ACT GGC
161    G   I   S   A   L   V   S   F   L   P   I   M   M   H   W   W   R   D   T   G

541    GAC CCC CTG GCC ATG AAA TGT TAC GAG GAT CCT GGG TGC TGT GAT TTT GTC ACC AAC AGA
181    D   P   L   A   M   K   C   Y   E   D   P   G   C   C   D   F   V   T   N   R

601    GCT TAC GCC ATC GCC TCG TCC ATC ATC TCC TTC TAT TTC CCA CTC ATC ATC ATG ATC TTC
201    A   Y   A   I   A   S   S   I   I   S   F   Y   F   P   L   I   I   M   I   F

661    GTC TAC ATC AGG GTC TTC AAG GAG GCG CAG AAG CAG ATG AAG AAG ATT GAC AAG TGC GAG
221    V   Y   I   R   V   F   K   E   A   Q   K   Q   M   K   K   I   D   K   C   E

721    GGC AGG TTC TCC CAT AGC CAC GTC CTG AGC CAC GGC AGG TCC AGC CGG AGG ATC CTC TCC
241    G   R   F   S   H   S   H   V   L   S   H   G   R   S   S   R   R   I   L   S

781    AAA ATC CTG GTG GCC AAA GAG CAG AAA GCC TTG AAG ACC CTC GGG ATT ATC ATG GGC ACC
261    K   I   L   V   A   K   E   Q   K   A   L   K   T   L   G   I   I   M   G   T

841    TTC ACC CTG TGC TGG TTG CCC TTC TTC TTG GCC AAC GTG GTC AAT GTC TTC TAC AGG AAC
281    F   T   L   C   W   L   P   F   F   L   A   N   V   V   N   V   F   Y   R   N

901    CTG ATC CCA GAC AAA CTC TTC CTC TTC CTC AAC TGG CTG GGC TAC GCC AAC TCC GCG TTT
301    L   I   P   D   K   L   F   L   F   L   N   W   L   G   Y   A   N   S   A   F

961    AAC CCC ATC ATC TAC TGC AGG AGC CCA GAC TTC AGG AAG GCT TTC AAG AGA CTC CTG TGT
321    N   P   I   I   Y   C   R   S   P   D   F   R   K   A   F   K   R   L   L   C

1021   TGC CCC AAA AAG GCA GAT CGG CAC CTC CAC ACT ACT GGG GAG CTC TCC CGA TAC TCG GGG
341    C   P   K   K   A   D   R   H   L   H   T   T   G   E   L   S   R   Y   S   G

1081   GGC TTT GTT AAC TCT TTA GAC ACC AAT GCT TTG GGT ATG TGT TCT GAA TGT AAT GGG GTG
361    G   F   V   N   S   L   D   T   N   A   L   G   M   C   S   E   C   N   G   V

1141   CGG ACG TCA TTG GAC TGA AAT TAA TTA TTT ATT GTG GGT CGG AGG GAG ATT GAA TAA GTG
381    R   T   S   L   D   *

1201   GGT GCG GGG CCC CAA ATA ACA GGT AGG TTC CAG GCA ACC TCA CTG CAG ATT CTT GGA ATG
1261   TAG AGG GTT CCC CAG GAT AGG AGT
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Fig. 1. Nucleotide and deduced amino acid sequence of the open reading frame of *X-β1AR* cDNA. The positions of the starting methionine and stop codon are designated by M and * respectively. Numbers refer to the cDNA sequence (+1 corresponding to the ATG codon) and the derived amino acid sequence.

3.2. Sequence analysis of X-β1AR protein

EMBL and GenBank database searches revealed that the whole amino acid sequence displays significant homology to those of the different subtypes of β-adrenergic receptors (Fig. 2). The percentages of identical amino acids in the sequence of X-β1AR compared to those of the human β_1-adrenergic receptor [28], the human β_2-adrenergic receptor [29] and the human β_3-adrenergic receptor [30] are 66%, 55%

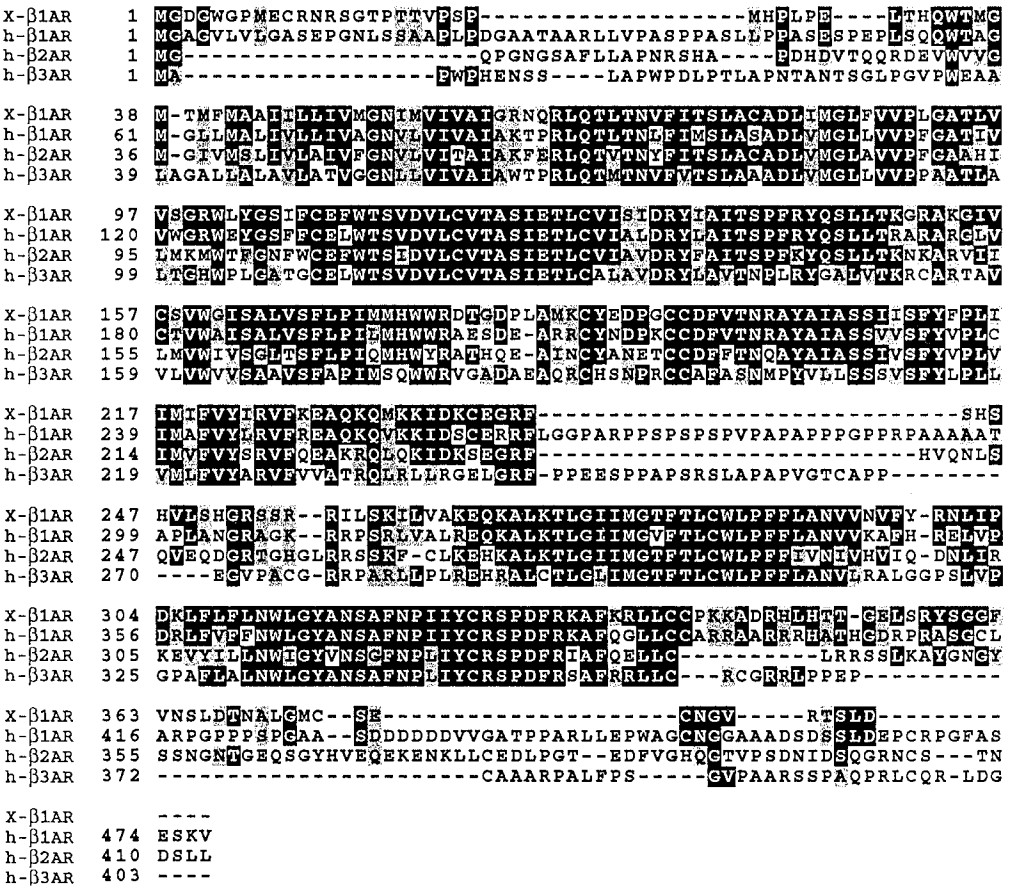

Fig. 2. Sequence analysis of the X-β1AR protein. The predicted amino acid sequence of X-β1AR was compared with that of the three subtypes of β-adrenergic receptors cloned from human species. Global alignment of the sequences of amphibian X-β1AR receptor, human β_1-adrenergic receptor [28], human β_2-adrenergic receptor [29] and human β_3-adrenergic receptor [30] was made with the Clustal V program. Identical residues are indicated by black boxes.

and 46%, respectively. Consequently, the higher degree of homology observed between the amphibian sequence and the human β_1-adrenergic receptor [28] strongly suggests that we have cloned the amphibian homologue of the β_1-adrenergic receptor.

3.3. *Expression of X-β1AR mRNA in Xenopus oocytes and embryos*

Expression of the *X-β1AR* gene was undetectable by Northern blot analysis. The low abundance levels of *X-β1AR* transcripts led us to use a RT-PCR assay for studying the temporal expression of *X-β1AR* mRNA during early embryogenesis. As shown in Fig. 3, the level of *X-β1AR* mRNA changes significantly during early development. An amplified fragment of the expected size (984 bp) was present in the oocyte. However, the amount of amplified fragments decreases significantly up to stage 6. From stage 8, mRNA levels gradually increase to reach high levels at larval stages. The constancy of amounts and loading of reverse-transcribed cDNAs was judged by PCR amplification with primers located in the sequence of the β_1 subunit of heterotrimeric GTP-binding proteins (Fig. 3) whose expression does not vary in the early stages of development [20]. These results clearly indicate that *X-β1AR* mRNA represents a maternal RNA whose levels gradually decline after fertilization up to stage 6. At the mid-blastula transition, the activation of the zygotic genome might

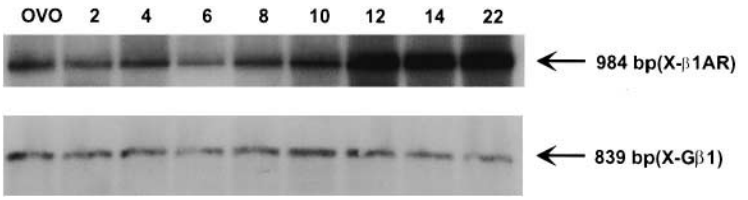

Fig. 3. Kinetics of *X-β1AR* gene expression. The upper part of the figure corresponds to amplification of the *X-β1AR* cDNA with primers corresponding to nucleotides 584–603 and 1540–1565. The lower part of the figure corresponds to amplification of the *X-Gβ1* cDNA with primers located at positions 183–206 and 1002–1021. Total RNAs were isolated from unfertilized eggs (ovo) or staged embryos and reverse-transcribed as described in Section 2. Amplified fragments were loaded on a 1% agarose gel. The dried gel was probed with the same amplified fragments which were randomly labeled with [^{32}P]α-dCTP. The expected size of each amplified fragment is indicated.

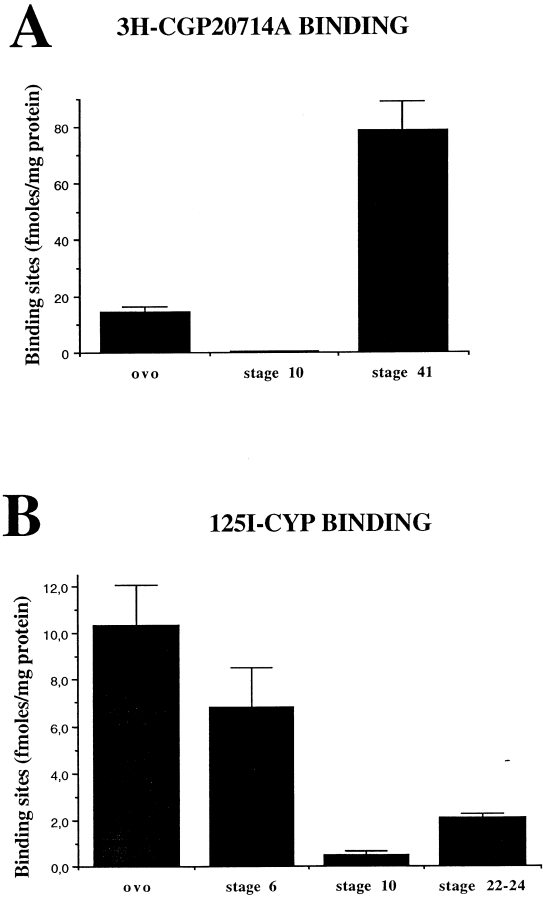

Fig. 4. Measurement of X-β1AR binding sites. A: Direct measurement by [^{3}H]CGP 20714A. Membranes were prepared from oocytes and staged embryos as described in Section 2. Membranes were incubated with 5 nM [^{3}H]CGP 20714A in the absence (total binding) or presence of 1 μM propranolol (non-specific binding). The specific binding corresponds to the propranolol-displaced binding. The values represent the mean of triplicate determinations from a representative experiment. B: Indirect measurement by the CGP 20714A-induced displacement of [^{125}I]cyanopindolol binding. Membranes were incubated with 150 pM [^{125}I]cyanopindolol in the absence (total binding) or presence of 1 μM CGP 20714A (non-specific binding). The specific binding corresponds to the binding displaced by CGP 20714A. The values represent the mean of triplicate determinations from a representative experiment.

be responsible for the increased mRNA levels observed after stage 8.

3.4. *Expression of X-β1AR protein in Xenopus oocytes and embryos*

In order to determine whether the protein and mRNA levels are correlated during early development, we first measured the number of binding sites at a saturating concentration of [^{3}H]CGP 20714A, a specific agonist of β_1-adrenergic receptors. Because of the large numbers of oocytes and embryos required for preparing the membranes used in binding studies, we first chose three representative developmental stages: the mature oocyte, the gastrula stage and a late larval stage. As shown in Fig. 4A, the oocyte contains an intermediate number of binding sites, whereas the gastrula displays a very low number of sites; on the other hand, the late larva possesses a large number of β_1-adrenergic receptors.

Because the low specific activity of the tritiated ligand (46 Ci/mmol) did not facilitate the accurate determination of such a low number of binding sites, we used an indirect approach involving the displacement of a saturating concentration of [^{125}I]CYP, a 'universal' β antagonist, in the presence of unlabeled CGP 20714A. To better examine the temporal variations, we narrowed the time interval between the different developmental stages that we analyzed before and after gastrulation. As shown in Fig. 4B, the numbers of binding sites in the oocytes and stage 10 embryos are very similar to those determined by the direct binding of the β_1 agonist. In addition, they decrease in stage 6 embryos, reaching the lowest levels of stage 10 embryos, to increase again in the early larval stage (stages 22–24).

Consistent with the RNA result, the level of X-β1AR protein is high in the oocyte and begins to decrease gradually upon fertilization. However, compared to the RNA, X-β1AR protein decreases at a slower rate and is still detectable at stage 6. This does not necessarily reflect a higher sensitivity of binding studies compared to PCR amplification, but rather suggests that the X-β1AR protein is more stable than the mRNA and remains present after the disappearance of the latter.

It should be pointed out that the binding assay not only measures the amount of protein but also examines its function, at least at the level of extracellular signal recognition.

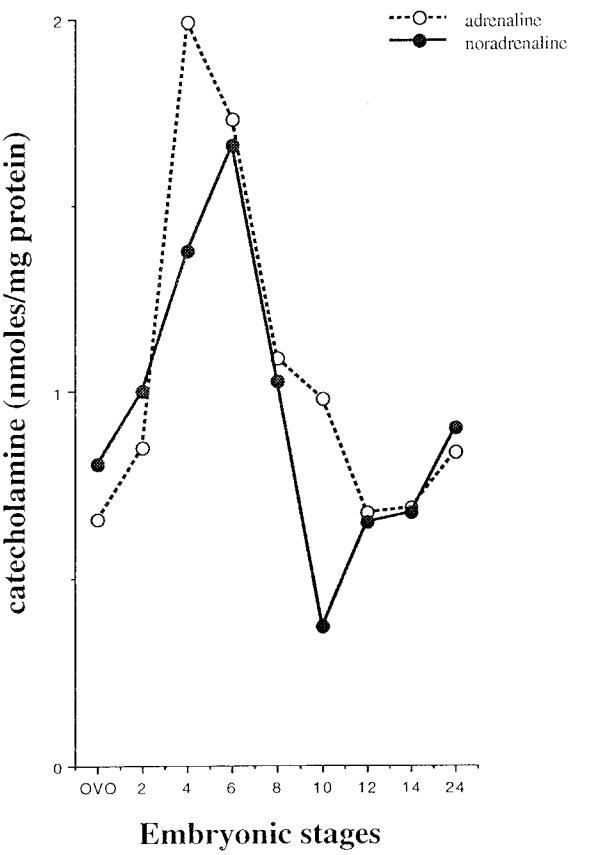

Fig. 5. Catecholamine content of oocytes and embryos. Ten oocytes or staged embryos were homogenized and their catecholamine levels determined using HPLC analysis as described in Section 2. Noradrenaline and adrenaline amounts are given in nmol/mg protein. Values represent the determination from pooled embryos and were obtained in a representative experiment.

3.5. Detection of noradrenaline in Xenopus oocytes and embryos

At all the stages that were assayed, noradrenaline and adrenaline were detected (Fig. 5). Significant levels of noradrenaline and adrenaline were also observed in the oocyte. The highest levels were found at stage 4 for adrenaline and at stage 6 for noradrenaline. The lowest amounts were observed at gastrulation, between stages 10 and 12. Subsequently, levels of both catecholamines increased again during early larval stages.

4. Discussion

We have shown the presence of mRNA encoding β_1-adrenergic receptors, characterized the existence of β_1-adrenergic binding sites and detected their putative ligands in early *Xenopus* embryos.

As far as β_1-adrenergic receptors are concerned, we have demonstrated the presence of maternal mRNAs before the mid-blastula transition which are relayed by the synthesis of zygotic mRNAs whose amount increases during neurulation. Similarly, β_1-adrenergic binding sites were also characterized but the decrease of the maternal protein as well as the increase of the zygotic protein were delayed when compared to those of the mRNA. Such a temporal difference could be explained by a differential turn-over of each species or a translational regulation. Interestingly, targeted disruption of the mouse β_1-adrenergic receptor gene is associated with a significant prenatal lethality and impaired cardiac performance [31]. As death occurs in late embryos, these data reveal that the β_1-adrenergic receptors required at a late stage of mouse development correspond to the zygotic receptors expressed in *Xenopus* embryos. On the other hand, these results do not address the question of the role played by the maternal β_1-adrenergic receptors.

Whatever the function of maternal receptors may be, it is noteworthy that we were able to detect the concomitant presence of catecholamines in oocytes and early embryos. Not only did we detect significant amounts of dopamine (data not shown), like Rowe et al. [32], but also low levels of noradrenaline and adrenaline, before the early neurula stage. The early detection of these catecholamines is doubtless explained by the high sensitivity of the electrochemical sensor. Interestingly, the decrease of catecholamine content between stages 6 and 10 strongly parallels that of the number of β_1-adrenergic binding sites. Although the presence of catecholamines in oocytes does not imply their de novo synthesis, the observed increase at stages 4 and 6 tends to suggest the existence of the enzyme machinery for such synthesis. Indeed, targeted disruption of mouse tyrosine hydroxylase [33] and dopamine β-hydroxylase [34] genes results in gestational lethality, with the same histological phenotype, due to cardiovascular failure. Again, these data correspond to the phase of catecholamine synthesis starting after the mid-blastula transition and cannot reveal the function of maternal catecholamines.

Since we started this work, recent reports have largely confirmed the validity of the general hypothesis that G protein-coupled receptors are expressed early in development and play a role in embryonic cell interactions. Several papers describe the characterization of putative G protein-coupled receptors for two signaling molecules implicated in numerous patterning events during development: Dfz2, a candidate wingless receptor [35], and Smo, a candidate hedgehog receptor [36,37]. A new G protein-coupled receptor is expressed in endothelial precursor during cardiogenesis and vasculogenesis [22]. Finally, serotonin receptors displaying the pharmacological properties of the mammalian 5-HT2 subtype are highly expressed during early *Drosophila* embryogenesis [38].

Interestingly, like the catecholamines, serotonin is mostly known for its neurotransmitter function in the mature central nervous system. Thus, besides the characterization of serotonin-like receptors, the concomitant presence of serotonin and of its complete machinery of synthesis strongly emphasizes a new role for serotonin signaling and transduction pathways in the decoding of early developmental signals in *Drosophila* [38]. In this context, the concomitant detection of noradrenaline and adrenaline as well as the expression of β_1-adrenergic receptors in *Xenopus* embryos tend to suggest that such an early developmental role for biogenic amines is not restricted to serotonin and that catecholamine signaling pathways might exert novel developmental functions in addition to their known role in differentiated cells of adult tissues.

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