Activation of the mouse oxytocin promoter by the orphan receptor ROR α

K Chu and H H Zingg

Laboratory of Molecular Endocrinology, Royal Victoria Hospital Research Institute, McGill University, Montreal, Quebec, Canada H3A 1A1

(Requests for offprints should be addressed to H H Zingg, Laboratory of Molecular Endocrinology, 687 Pine Avenue West, Room H7.63, Montreal, Quebec, Canada H3A 1A1; Email: zingg@rvhri.lan.mcgill.ca.)

ABSTRACT

Although an increasing number of nuclear orphan receptors have recently been identified, the number of known naturally occurring genes that are directly regulated by orphan receptors is still small. We have shown previously that the gene encoding the neuropeptide oxytocin (OT) is negatively regulated by the orphan receptors chicken ovalbumin upstream transcription factor I (COUP-TFI) and II. Here we show that the mouse OT gene promoter is activated by ROR α , a representative of the ROR/RZR orphan receptor subfamily. Using promoter/chloramphenicol acetyltransferase reporter constructs in heterologous transfection assays, we determined that ROR α action induces a <6-fold increase in promoter activity. By 5' and 3' deletion analysis, DNase footprint analysis and

electrophoretic mobility shift assays, we found that ROR α action is mediated by two 14 bp regions centered at 160 and 180 nucleotides upstream of the transcriptional initiation site. Both sites contain significant sequence identities with an established ROR recognition sequence. Mutations in either or both of these sites reduce significantly ROR α -induced activation of the OT promoter. In view of the strong transcriptional activation exerted by ROR α on the OT gene promoter and the widespread distribution of different members of the ROR/RZR family, interactions between ROR/RZR isoforms and the OT gene may form part of the multifactorial regulatory mechanisms that control OT gene expression in different tissues.

Journal of Molecular Endocrinology (1999) **23**, 337–346

INTRODUCTION

The nonapeptide hormone oxytocin (OT) acts as a neurotransmitter as well as a peripheral hormone. Whereas central effects of OT involve induction of specific reproductive behaviors, peripheral effects include uterine contractions, milk ejection, natriuresis and vasodilation (Zingg 1996, Thibonnier *et al.* 1999). Centrally, the OT gene is expressed in specific hypothalamic neuroendocrine cells as well as in the pineal gland (Milcu *et al.* 1963, Fisher & Fernstrom 1981, Liu & Burbach 1987). Peripherally, the gene is expressed in uterine epithelium (Lefebvre *et al.* 1992), fetal membranes (Mitchell *et al.* 1998) and the corpus luteum (Ivell *et al.* 1998). OT gene expression is hormonally induced by estrogen and retinoic acid (Larcher *et al.* 1995). In addition, we and others have shown that orphan nuclear receptors, specifically members of the chicken ovalbumin upstream transcription

factor (COUP-TF) family, antagonize hormone-mediated transcriptional activation of the OT gene and actively silence the OT gene promoter (Burbach *et al.* 1994, Chu & Zingg 1997, Chu *et al.* 1997). These nuclear receptor/promoter interactions are mediated by a well-conserved region centered at –160 relative to the transcriptional initiation site (Richard & Zingg 1990, Adan *et al.* 1993). This region was initially identified as an estrogen response element (ERE) (Richard & Zingg 1991) and is here referred to as OT/ERE.

In the present study, we have examined the role of the recently identified orphan receptor ROR α in the regulation of the OT promoter. The ROR/RZR subfamily of orphan nuclear receptors is a recent addition to the nuclear receptor family and comprises the following members: ROR α 1, ROR α 2, ROR α 3, ROR β /RZR β and ROR γ (Carlberg *et al.* 1994, Giguère *et al.* 1994, Hirose *et al.* 1994). The members of this subfamily bind as monomers

preferentially to an AGGTCA core motif preceded by an A/T-rich region (Giguère *et al.* 1994). Although the expression pattern of this receptor family is quite ubiquitous, high expression of specific members is observed in certain tissues. Thus, ROR α 1 is highly expressed in Purkinje cells of the mouse cerebellum (Matsui *et al.* 1995), and a mutation in the ROR α 1 gene gives rise to the *staggyr* mouse phenotype (Hamilton *et al.* 1996, Dussault *et al.* 1998). ROR β /RZR is abundantly expressed in the hypothalamus, the pineal gland and the thalamus (Becker-Andre *et al.* 1994). ROR γ is highly expressed in skeletal muscle and may play a role in muscle development (Hirose *et al.* 1994).

Although structural information on the orphan receptor superfamily is rapidly expanding, relatively few genes have been identified that are directly regulated by orphan receptors. Identified target genes for members of the ROR/RZR family include the gene for γ F-crystallin, apolipoprotein A-I, 5-lipoxygenase, cellular retinol-binding protein, retinoic acid receptor- β , p21^{WAF/CIP1} and Purkinje cell protein-2 (Steinhilber *et al.* 1995, Tini *et al.* 1995, Schröder *et al.* 1996, Vu-Dac *et al.* 1997). Here we report that the mouse OT gene represents a novel target for ROR receptors by demonstrating that its promoter is strongly stimulated by ROR α via a DNA element that has been conserved throughout species evolution.

MATERIALS AND METHODS

Plasmids

Plasmid p1064CAT contained nucleotides -1064 to $+38$ of the mouse OT promoter (Hara *et al.* 1990) linked to the gene encoding chloramphenicol acetyltransferase (CAT). The deletion mutants p428CAT, p225CAT, p118CAT and p42CAT were derived from p1064CAT. p225 Δ OT/ERE, p225 Δ HS1 and p225 $\Delta\Delta$ mutants were obtained by site-directed mutagenesis (Kunkel 1985). The mutagenic oligonucleotides were also used in the gel shift assays (see below). Plasmids p225/118-TATACAT and p134/34-TATACAT contained fragments -225 to -118 and -134 to -34 respectively of the mouse OT 5'-flanking region linked to a TATA-box derived from the adenovirus 1b followed by the gene encoding CAT. The parent plasmid β -CAT containing the TATA-box linked to the CAT gene was obtained from Dr V Giguère, (Royal Victoria Hospital, McGill University, Montreal). All constructs were verified by dideoxynucleotide sequencing. Expression vectors CMX and CMX-ROR α 1 were also obtained from Dr Giguère (Giguère *et al.* 1994). These contained a

cytomegalovirus-derived promoter which, in CMX-ROR α 1, was linked to a cDNA encoding mouse ROR α 1. The vector HSP- β -gal contained a heat shock protein (HSP) gene-derived promoter linked to the β -galactosidase gene (obtained from Dr Giguère).

Transfections

Mouse Neuro-2a cells (American Type Culture Collection, Rockville, MD, USA; line CCL 131) were maintained in Eagle's minimum essential medium (EMEM; Sigma, St Louis, MI, USA) supplemented with 10% fetal bovine serum (GIBCO, Rockville, MD, USA). Cells were plated on 60 mm dishes and allowed to reach 70–80% confluency before transfection. Plasmids used in transfection studies were purified twice by ultracentrifugation through 5.2 M cesium chloride. Each plate was transfected with a total of 10 μ g DNA, including 5 μ g CAT reporter plasmid, 3 μ g expression vectors, 100 ng HSP- β galactosidase plasmid and 1.9 μ g pBluescript plasmid KS(+). Transfection was performed by the calcium phosphate precipitation technique. After 4 h of incubation with the precipitate, a glycerol shock was applied by incubating cells in a buffered saline solution containing 15% glycerol for 2.5 min. Cells were then washed with PBS and cultured in EMEM/10% fetal bovine serum. Forty-eight hours after the transfection, cells were collected and CAT activity was determined, using a phase extraction method (Seed & Sheen 1988). Unless stated otherwise, transfections were performed in duplicates at least three times. CAT activity was normalized with respect to β -gal activity recovered from cell extracts. Cotransfection with the ROR α 1 expression vector remained without effect on the transcriptional activity of the HSP- β -gal expression vector.

Bacterial expression of proteins

Recombinant human ROR α 1 and rat COUP-TFII were produced in BL21 cells as glutathione-S-transferase (GST)-fusion proteins. The cDNAs encoding human ROR α 1 or rat COUP-TFII were amplified by PCR and inserted in-frame with the GST-coding region in the bacterial expression plasmid pGEXKG (Novagen, Madison, WI, USA). The resulting plasmid was used to transform BL21 cells as described (Novagen). Cells were grown until they reached an OD₅₅₀ of 0.6 and Isopropyl- β -D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM. Three hours after IPTG induction, cells were collected and purified using a glutathione resin (Pharmacia, Uppsala,

Sweden), according to the manufacturer's instructions. Finally, the GST moiety was removed by thrombin cleavage

DNase I footprinting assays

Probes for DNase footprinting experiments were generated by PCR. The primer pair used for the amplification reaction consisted of oligonucleotide R48 (complementary to a part of the pBluescript polylinker region, sense strand direction) and a reverse primer complementary to the 5'-end of the CAT cDNA (anti-sense direction). The oligonucleotide R48 was labeled with ^{32}P using [^{32}P] γ ATP and T4 polynucleotide kinase. For the PCR reaction, p225CAT was used as a template in conjunction with 10 pmol of each primer. Following 35 cycles of amplification (94 °C, 1 min; 58 °C, 1 min; 72 °C, 1 min) the PCR product was gel purified and diluted to 10 000 c.p.m./ml. For DNase I footprinting analysis, 0.06–5 ml of recombinant protein were incubated with 10 000 c.p.m. DNA probe in binding buffer (12 mM Hepes (pH 7.9) 12% glycerol, 70 mM KCl, 3 mM MgCl_2 , 250 ng poly(dI-dC); Pharmacia). Following incubation of the binding reaction for 30 min at room temperature, 0.25 unit of DNase I (RQ1-DNase I, Promega, Madison, WI, USA) was added for 90 s. The endonuclease reaction was stopped by the addition of EDTA to a final concentration of 5 mM. The binding reaction was phenol extracted, precipitated with ethanol and resuspended in 90% formamide loading buffer. Equal amounts of recovered probe were then loaded on a 10% denaturing polyacrylamide gel.

Electrophoretic mobility shift assay (EMSA)

For EMSAs, *in vitro* translated CMX or CMX-ROR α 1 was used. Translation products were obtained *in vitro* by using plasmids CMX or CMX-ROR α as templates in an *in vitro* transcription/translation reaction mix (TNT lysate, Promega) according to the manufacturer's instruction. All proteins were [^{35}S]methionine labeled and their sizes verified by electrophoresis in 10% SDS-polyacrylamide gels.

Ten picomoles double-stranded oligonucleotides were labeled using T4 kinase and [^{32}P] γ ATP. Two milliliters of *in vitro* translated ROR α 1 were incubated in binding buffer with 10 fmol probe for 30 min at room temperature and electrophoresed on a non-denaturing 4% polyacrylamide gel in 0.25 $\times$ TBE buffer at 150 V. A polyclonal antibody against rat ROR α 1 was obtained from Dr V Giguère (Dussault *et al.* 1998).

RESULTS

We tested the effect of ROR α 1, a member of the family of orphan receptors, on OT promoter activity by transfecting 5' deletion mutants of mouse OT-CAT reporter plasmids in mouse Neuro-2a cells in conjunction with an expression plasmid for ROR α 1 (CMX-ROR α 1). With the longest reporter construct used (p1064CAT), cotransfection of the ROR α 1 expression vector CMX-ROR α 1 led to a 6- to 7-fold increase in CAT activity compared with cells transfected with the parental plasmid CMX (Fig. 1). Similar or greater activation was also observed with shorter constructs (p428CAT and p225CAT). However, with the construct p118CAT, ROR α 1 elicited only a 2.2-fold increase in activity. No significant activation was observed with the construct p42CAT. These studies indicate that the sequence interval –225 and –42 harbors a region involved in conferring inducibility by ROR α .

To delineate further the 3'-end of the area mediating this response, regions –225 to –118 (p225/118TATA-CAT) and –134 to –34 (p134/34TATA-CAT) were cloned upstream of a minimal adenovirus 1b TATA-box/CAT reporter and transfected in Neuro-2a cells (Fig. 2). Cotransfection of ROR α 1 elicited a strong 14-fold increase in CAT activity mediated by the construct p225/118TATA-CAT. Only a very weak effect was observed with p134/42TATA-CAT or the parental reporter construct (β -CAT). These results narrow the area mediating activation by ROR α 1 further down to the region –225 to –118.

DNase I footprinting with bacterially expressed ROR α 1 was used to identify more precisely the regions involved in ROR α 1 binding. Two adjacent regions were found to be resistant to DNase I digestion following incubation with ROR α 1 (Fig. 3). One region was centered at position –180 and the other at position –160. The first region (–188 to –174) includes the sequence element AGGTCA. As shown by Umesono *et al.* (1991), this element forms the building block of several types of nuclear receptor response elements (see Discussion). The second region encompasses the OT/ERE and contains three AGGTCA-related motifs, which can be viewed either as an inverted repeat (HS2 and HS3) or as a direct repeat (HS3 and HS4) (Fig. 4A). The center of the footprint appeared to be located over HS4 (Fig. 3).

For comparison, the binding of the orphan receptor COUP-TFII to this same promoter area was also examined. COUP-TFII is a prototype for dimer binding. We have previously shown that COUP-TFII binds to the human OT/ERE (Chu *et al.* 1997). Lane 4 in Fig. 3 shows that bacterially

FIGURE 1. Activation of the mouse OT promoter by RORα1. (A) Schematic diagram of constructs used. Different length segments of the 5'-flanking region of the mouse OT gene were linked to the structural gene for CAT. Sequence positions are indicated with respect to the transcriptional initiation site. (B) The constructs shown in (A) were transfected in mouse Neuro-2a cells together with a lacZ expression vector and the parent expression vector CMX or the RORα1 expression vector CMX-RORα. CAT activity was determined in cell extracts and was normalized with respect to β-gal activity. RORα1-mediated transcriptional activation was expressed as the ratio of CAT activity in cells transfected with vector CMX-RORα vs cells transfected with the parent expression vector CMX. Each bar represents the mean ± S.E. of three or more independent experiments, each performed in duplicate.

expressed COUP-TFII also binds to the mouse OT/ERE and HS1, but the regions protected by COUP-TFII are larger than the ones protected by RORα1. This is compatible with the accepted concept that COUP-TF binds as a dimer, whereas RORα binds as a monomer. Indeed, COUP-TF homodimers have been shown to bind with high affinity to monomeric ROR/RZR response elements (Schröder *et al.* 1996). In addition, COUP-TFII is also able to bind to a more downstream area (– 75

to – 100) containing three (inverted) GGTCA repeats (Fig. 3) as demonstrated earlier for the human gene (Chu *et al.* 1997). A noticeable but weak footprint is also formed by RORα in this region.

EMSA was performed to further characterize RORα1 binding to the OT/ERE and the HS1. *In vitro* translated RORα1 protein was incubated with oligonucleotides encompassing either the OT/ERE (Fig. 4B) or the HS1 (Fig. 4C). RORα1 readily

FIGURE 2. The region -225 to -118 of the OT promoter is sufficient for ROR α 1-mediated activation. (A) Schematic diagram of constructs used. Regions -225 to -118 (p225/118TATA-CAT) and -134 to -34 (p134/34TATA-CAT) were cloned upstream of the adenovirus 1b TATA-box and transfected into Neuro-2a cells together with the vector CMX or CMX-ROR α 1. (B) CAT activity was normalized with respect to β -gal activity and was expressed as fold induction observed in CMX-ROR α -transfected cells over CMX-transfected cells, as in Fig. 1. Each bar represents the mean $\pm$ S.E. of three or more independent experiments, each performed in duplicate.

bound specifically to the OT/ERE (Fig. 4B, lanes 3–6). In contrast to the wild-type OT/ERE oligonucleotide, an oligonucleotide carrying a mutated OT/ERE (Δ OT/ERE) was unable to compete for binding (lanes 4–6 vs 7–9). ROR α 1 binding to the OT/ERE probe was also competed by an oligonucleotide containing HS1 (lanes 10–12). The consensus RORE represented an even stronger competitor. Whereas a 25-fold excess of cold RORE oligonucleotide readily competed away binding of

the OT/ERE or the HS1 probes, a 100-fold excess of homologous cold probe was required to observe the same effect (Fig. 4B and C).

We next tested mutations that abrogate ROR α binding with respect to their effect on promoter function. The construct p255 Δ OT/ERE-CAT carried the mutation present in the oligonucleotide Δ OT/ERE used in the EMSA studies above. Similarly, the mutations in construct p225 Δ HS1-CAT corresponded to the mutations in the

FIGURE 3. DNaseI footprinting analysis of RORα1-binding sites on the mouse OT gene promoter. Recombinant human RORα1 (lane 3) and rat COUP-TFII (lane 4) were used in conjunction with a 5'-end (sense strand)-labeled 320 bp OT promoter fragment. BSA: BSA was used instead of recombinant protein. G+A: Maxam-Gilbert sequencing ladder (G+A reaction). Relevant sequence portions are indicated on the left (sense strand).

oligonucleotide ΔHS1. Finally, the construct p255ΔΔ-CAT contained the combined mutations of ΔHS1 and ΔOT/ERE. Whereas wild-type

p225CAT activity was stimulated 9-fold by RORα1, the mutant p225ΔOT/ERE-CAT was only stimulated 4-fold (Fig. 5). Similarly, construct p225ΔHS1-CAT was only induced 3-fold (Fig. 5). Introduction of a double mutation (p225ΔΔ-CAT) led to a further decrease in inducibility to about 2-fold. These results suggest that the OT/ERE and the HS1 sites both contribute additively, but not synergistically, to RORα1-mediated induction.

DISCUSSION

We and others have shown that selected members of the nuclear receptor family are potent regulators of OT expression. This includes the nuclear receptors for estrogens, retinoids and thyroid hormone, as well as the orphan receptors COUP-TFI, COUP-TFII, Erb-A-related receptor 2 (Erb-2) and steroidogenic factor 1 (SF-1) (Richard & Zingg 1990, 1991, Adan *et al.* 1992, 1993, Burbach *et al.* 1994, Wehrenberg *et al.* 1994). Here we demonstrate that a member of the orphan receptor family ROR/RZR acts as a regulator of OT expression. In contrast to all other orphan receptors that have so far been shown to act as inhibitors or, in the case of SF-1, as only weak activators of the OT gene promoter (Wehrenberg *et al.* 1994), RORα1 acts as a strong activator of the OT gene promoter.

We have demonstrated that the OT promoter is stimulated by the orphan receptor RORα1 via a small region of the promoter which can be transferred to a heterologous adenovirus 1b TATA promoter. This small region contains the conserved OT/ERE and an AGGTCA half-site (HS1) located 5' of the OT/ERE. Mutation of any of these elements leads to reduced activation by RORα1. In addition, using DNase I footprinting and EMSA experiments, RORα1 was shown to bind specifically to these two elements.

By PCR-mediated site selection, Giguère *et al.* (1994) have determined a consensus sequence for RORα1 binding as follows: (A/G/T)^T/A^A/T^T/A^C/A AGGTCA (Fig. 6). The OT/ERE contains three half-sites, HS2, HS3 and HS4 (Fig. 6). According to the results from our footprinting studies, HS4 seems to be preferentially recognized by RORα1. Comparison of HS4 with the consensus RORα1-binding site indicates that, in addition to the conserved AGGTCA half-site, nucleotides -1, -2 and -3 preceding the AGGTCA also conform to the consensus binding site (Fig. 6). Nucleotide -4, however, differs from the consensus sequence. Comparison of the HS1 with the consensus RORα1-binding site reveals that positions -2, -3, -4 and -6 relative to the AGGTCA motif all

FIGURE 4. EMSAs of RORα1 binding to the OT/ERE and mutants thereof. (A) Double-stranded oligonucleotides used in EMSA. The OT/ERE with its intrinsic half-sites (HS2, HS3 and HS4) as well as the upstream half-site HS1 are shown. (B) *In vitro* transcribed/translated RORα1 (lanes 2–15) or unprogrammed reticulocyte lysate (lane 1) was incubated with ³²P-labeled OT/ERE double-stranded oligonucleotide. Lane 3: coinubation with an anti-RORα1 antibody. Binding was competed with increasing concentrations (5-, 25- and 100-fold excess) of unlabeled homologous oligonucleotide OT/ERE (lanes 4–6), ΔOT/ERE (lanes 7–9), HS1 (lanes 10–12), or ROR response element (RORE) (lanes 13–15). (C) As in (B) but the probe HS1 was used instead of ΔOT/ERE.

conform to the consensus sequence. Of note is the presence of a C at position –2 which is conserved throughout in the OT/ERE, the HS1 and the RORα1 consensus binding sequence.

Mutation of the OT/ERE or HS1 that abolished binding to RORα1 reduced RORα1-mediated induction by 56 and 68% respectively and a combined mutation of both the OT/ERE and the

FIGURE 5. Effect of mutations in OT/ERE and HS1 on activation by RORα1. (A) Schematic representation of constructs used. Mutations were the same as in Fig. 4 and are denoted by 'x'. Half-sites are indicated by arrows. (B) Constructs shown in (A) were cotransfected with the expression vector CMX or CMX-RORα1 in Neuro-2a cells and CAT activity was determined. RORα1-mediated transcriptional activation was expressed as the ratio of CAT activity in cells transfected with vector CMX-RORα vs cells transfected with the parent expression vector CMX. Each bar represents the mean ± s.e. of three or more independent experiments, each performed in duplicate.

FIGURE 6. (A) Region -145 to -195 of the mouse OT gene promoter. HS1 and the OT/ERE, containing HS2, HS3 and HS4, are indicated. Arrows indicate the AGGTCA-related half-sites. (B) Comparison of the HS4 of the OT/ERE and HS1 with the consensus RORE. Upper and lower case letters indicate matches and mismatches respectively.

HS1 lowered the induction by 76% (Fig. 5B). The residual activity of the double mutant is probably due to the downstream AGGTCA repeats, a region which is also weakly protected in the DNase I footprinting assay.

It remains to be determined to what extent other members of the ROR/RZR family have the same or similar effects on OT gene promoter activity. Studies by Carlberg *et al.* (1994) indicate that ROR α 1 and ROR β /RZR β have indistinguishable binding characteristics and suggest considerable functional overlap between these ROR/RZR isoforms. In view of the expanding list of isoforms present in the ROR/RZR family and the widespread distribution of these members, it is conceivable that interactions similar to the one characterized here for the case of ROR α 1 may take place in different OT-expressing tissues, involving different ROR/RZR isoforms. The strong, ligand-independent activation function of ROR α 1 demonstrated here suggests that ROR may form an important part of the multifactorial regulatory mechanisms that control the neuronal and non-neuronal expression of the OT gene.

ACKNOWLEDGEMENTS

We thank Dr J Battey, NIH, Rockville, MD, USA for supplying us with the plasmid p11.38 containing

the mouse OT gene and Dr V Giguère for giving us the vectors CMX-ROR α , HSP- β -gal and β -CAT as well as for valuable advice and discussion. We also thank Miss E Monaco for excellent secretarial assistance.

REFERENCES

- Adan RA, Cox JJ, Van Kats JP & Burbach JP 1992 Thyroid hormone regulates the oxytocin gene. *Journal of Biological Chemistry* **267** 3771-3777.
- Adan RA, Cox JJ, Beischlag TV & Burbach JP 1993 A composite hormone response element mediates the trans-activation of the rat oxytocin gene by different classes of nuclear hormone receptors. *Molecular Endocrinology* **7** 47-57.
- Becker-Andre M, Wiesenberg I, Schaeren-Wiemers N, Andre E, Missbach M, Saurat JH & Carlberg C 1994 Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily *Journal of Biological Chemistry* **269** 28531-28534.
- Burbach JP, Lopes da Silva S, Cox JJ, Adan RA, Cooney AJ, Tsai MJ & Tsai SY 1994 Repression of estrogen-dependent stimulation of the oxytocin gene by chicken ovalbumin upstream promoter transcription factor I. *Journal of Biological Chemistry* **269** 15046-15053.
- Carlberg C, Hooft van Huijsduijnen R, Staple JK, DeLamarier JF & Becker-Andre M 1994 RZR α s, a new family of retinoid-related orphan receptors that function as both monomers and homodimers. *Molecular Endocrinology* **8** 757-770.
- Chu K & Zingg HH 1997 The nuclear orphan receptors COUP-TFII and Ear-2 act as silencers of the human oxytocin gene promoter. *Journal of Molecular Endocrinology* **19** 163-172.

- Chu K, Boutin J-M, Breton C & Zingg HH 1997 Nuclear orphan receptors COUP-TFII and Ear-2: presence in oxytocin-producing uterine cells and functional interaction with the oxytocin gene promoter. *Molecular and Cellular Endocrinology* **137** 145–154.
- Dussault I, Fawcett D, Matthyssen S, Bader J-A & Giguere V 1998 Orphan nuclear receptor RORα-deficient mice display the cerebellar defect of staggerer. *Mechanisms of Development* **70** 147–153.
- Fisher LA & Fernstrom JD 1981 Measurement of nonapeptides in pineal and pituitary using reversed-phase, ion-pair liquid chromatography with post-column detection by radioimmunoassay. *Life Sciences* **28** 1471–1481.
- Giguère V, Tini M, Flock G, Ong E, Evans RM & Otulakowski G 1994 Isoform-specific amino-terminal domains dictate DNA-binding properties of ROR α, a novel family of orphan hormone nuclear receptors. *Genes and Development* **8** 538–553.
- Hamilton BA, Frankel WN, Kerrebrock AW, Hawkins TL, FitzHugh W, Kusumi K, Russell LB, Mueller KL, Van Berkel V, Birren BW, Kruglyak L & Lander ES 1996 Disruption of the nuclear hormone receptor RORα in staggerer mice. *Nature* **379** 736–739.
- Hara Y, Battey J & Gainer H 1990 Structure of mouse vasopressin and oxytocin genes. *Molecular Brain Research* **8** 319–324.
- Hirose T, Smith RJ & Jetten AM 1994 ROR gamma: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle. *Biochemical and Biophysical Research Communications* **205** 1976–1983.
- Ivell R, Bathgate RA, Walther N & Kimura T 1998 The molecular basis of oxytocin and oxytocin receptor gene expression in reproductive tissues. *Advances in Experimental Medicine and Biology* **449** 297–306.
- Kunkel TA 1985 Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proceedings in the National Academy of Sciences of the USA* **82** 488–492.
- Larcher A, Neculcea J, Chu K & Zingg HH 1995 Effects of retinoic acid and estrogens on oxytocin gene expression in the rat uterus: *in vitro* and *in vivo* studies. *Molecular and Cellular Endocrinology* **114** 69–76.
- Lefebvre DL, Giaid A, Bennett H, Lariviere R & Zingg HH 1992 Oxytocin gene expression in rat uterus. *Science* **256** 1553–1555.
- Liu B & Burbach JP 1987 Characterization of vasopressin and oxytocin immunoreactivity in the sheep and rat pineal gland: absence of vasotocin and detection of a vasopressin-like peptide. *Peptides* **8** 7–11.
- Matsui T, Sashihara S, Oh Y & Waxman SG 1995 An orphan nuclear receptor, mRORα, and its spacial expression in adult mouse brain. *Molecular Brain Research* **33** 217–226.
- Milcu SM, Pavel S & Neacsu C 1963 Biological and chromatographic characterization of a polypeptide with pressor and oxytocic activities isolated from the bovine pineal gland. *Endocrinology* **72** 563–566.
- Mitchell BF, Fang X & Wong S 1998 Oxytocin: a paracrine hormone in the regulation of parturition? *Reviews of Reproduction* **3** 113–122.
- Richard S & Zingg HH 1990 The human oxytocin gene promoter is regulated by estrogens. *Journal of Biological Chemistry* **265** 6098–6103.
- Richard S & Zingg HH 1991 Identification of a retinoic acid response element in the human oxytocin promoter. *Journal of Biological Chemistry* **266** 21428–21433.
- Schröder M, Danielsson C, Wiesenberg I & Carlberg C 1996 Identification of natural monomeric response elements of the nuclear receptor RZR/ROR. *Journal of Biological Chemistry* **271** 19732–19736.
- Seed B & Sheen JY 1988 A simple phase-extraction assay for chloramphenicol acetyltransferase activity. *Gene* **67** 271–277.
- Steinhilber D, Brungs M, Werz O, Wiesenberg I, Danielsson C, Kahlen J-P, Mayeri S, Schrader M & Carlberg C 1995 The nuclear receptor for melatonin represses 5-lipoxygenase gene expression in human B lymphocytes. *Journal of Biological Chemistry* **270** 7037–7040.
- Thibonnier M, Conarty D, Preston J, Plesnicher C, Dweik R & Erzurum S 1999 Human vascular endothelial cells express oxytocin receptors. *Endocrinology* **140** 1301–1309.
- Tini M, Fraser RA & Giguere V 1995 Functional interactions between retinoic acid receptor-related orphan nuclear receptor (ROR α) and the retinoic acid receptors in the regulation of the gamma F-crystallin promoter. *Journal of Biological Chemistry* **270** 20156–20161.
- Umesono K, Murakami KK, Thompson CC & Evans RM 1991 Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. *Cell* **65** 1255–1266.
- Vu-Dac N, Gervois P, Grotzinger T, De Vos P, Schoonjans K, Fruchart J-C, Auwerx J, Mariani J, Tedgui A & Staels B 1997 Transcriptional regulation of apolipoprotein A-I gene expression by the nuclear receptor RORα. *Journal of Biological Chemistry* **272** 22401–22404.
- Wehrenberg U, Ivell R, Jansen M, von Goedecke S & Walther N 1994 Two orphan receptors binding to a common site are involved in the regulation of the oxytocin gene in the bovine ovary. *Proceedings of the National Academy of Sciences of the USA* **91** 1440–1444.
- Zingg HH 1996 Vasopressin and oxytocin receptors. In *Bailliere's Clinical Endocrinology and Metabolism*, pp 75–96. Eds MC Sheppard & JA Franklyn. London: Bailliere Tindall.

RECEIVED 12 May 1999