Retinoic Acid Can Block Differentiation of the Myocardium after Heart Specification

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While a number of transcription factors that are likely to play a role in cardiac differentiation have recently been described, the signals that lead to the expression of these factors remains poorly understood. Here we report that exposure of Xenopus embryos to continuous low levels of all-trans retinoic acid (RA), starting at the time of neural fold closure, blocks expression of myocardial differentiation markers. The development of the remainder of the embryo is relatively normal, suggesting that retinoic acid can act rather specifically on myocardial precursors. Indeed, the pattern of endocardial gene expression appears to remain unaffected by RA treatment. Although RA blocks myocardial gene expression, a superficially normal heart tube forms. The heart tube, however, fails to loop during subsequent development and never forms beating tissue. The effect of RA treatment on expression of myocardial genes is developmental stage dependent, since no influence is observed after myocardial differentiation has commenced. These data indicate that a vital component of the myocardial determination pathway is sensitive to retinoid signaling.

INTRODUCTION

A growing number of transcription factors that play a role in early cardiac development have been identified. These include homeobox-containing genes related to Drosophila tinman (Lints et al., 1993; Tonissen et al., 1994; Schultheiss et al., 1995; Evans et al., 1995; Lee et al., 1996), members of the MADS box family (Edmondson et al., 1994), members of the GATA family (Kelley et al., 1993), and members of the bHLH family (Srivastava et al., 1995). However, the signaling processes that initiate the expression of these factors remain unknown. In Xenopus, embryological studies have determined that the heart precursor cells are specified, gaining the ability to express cardiac markers when cultured in a neutral environment, during gastrulation (Sater and Jacobson, 1989; Nascone and Mercola, 1995). Expression of several transcription factors known to be important for heart development commences during gastrulation (Kelley et al., 1993; Tonissen et al., 1994; Evans et al., 1995), providing further evidence that the cardiogenic program is initiated at this stage. Differentiation of the Xenopus heart begins much later, at the tailbud stage (stage 26/27), based both on changes in morphology (Nieuwkoop and Faber, 1994) and on the expression of cardiac-specific differentiation products (Logan and Mohun, 1993; Drysdale et al., 1994; Chambers et al., 1994).

Sater and Jacobson (1990) have shown that, in Xenopus, the precardiac tissue has characteristics of an embryonic field, meaning that the area of tissue first specified to become heart is larger than the area that will differentiate into heart. The process of generating a smaller heart-forming region does not appear to be due to migration of cardiac precursors. Instead, a signal that inhibits the cardiogenic program at the periphery of the heart field is likely to be responsible (Sater and Jacobson, 1990). The nature of this signal remains unknown. This restriction of the field may be related to the levels of the tinman-related genes Nkx2.5 and Nkx2.3. If the expression of these genes is elevated, a larger number of cells express cardiac differentiation markers and a larger heart results. It seems likely that the ele-
vated levels of these tinman-related proteins in the heart field act to resist the normal contraction of the heart field (Cleaver et al., 1996; Chen and Fishman, 1996).

We have identified a simple treatment using retinoids that causes a reduction in the expression of Nkx2.5 and Nkx2.3 and blocks differentiation of the myocardium. The retinoid family of molecules acts on a variety of biological functions through interactions with specific receptors and binding proteins (reviewed in Giguere, 1994). Exposure to exogenous retinoids is teratogenic for a wide variety of structures, and these structures appear to be most vulnerable during the period of specification (Shenefelt, 1972). In response to RA treatment, changes in cell fate are often observed (Kessel and Gruss, 1991). More specifically, a number of studies have shown that heart development is sensitive to exogenous retinoid treatment (Osmond et al., 1991; Stainier and Fishman, 1992; Yutzey et al., 1994; Drysdale et al., 1994). Recently, heart defects have been observed in mice lacking one or more specific retinoic acid receptors, indicating that some aspect of RA signaling is required for normal heart development (Sucov et al., 1994; Kastner et al., 1994).

In this paper, we demonstrate that exposure of Xenopus embryos to all-trans retinoic acid can completely block the differentiation of the myocardium. The exposure to RA is initiated after the time of heart specification and the effects are specific to the myocardium—the endocardium is not noticeably affected. If, however, retinoic acid treatment is initiated after myocardial differentiation has commenced, there is no discernible effect on subsequent heart development. We conclude that the process of myocardial determination is sensitive to retinoic acid and that this treatment may be mimicking a pathway that restricts the heart field during normal cardiogenesis.

MATERIALS AND METHODS

Embryo Manipulations

Xenopus laevis embryos were generated as in Drysdale and Elinson (1991) except that adult females were not primed with pregnant mare serum gonadotrophin. Embryos were dejellied in 2.5% cysteine, pH 8.0, and were staged according to Nieuwkoop and Faber (1994).

Embryos were treated with various concentrations of all-trans retinoic acid (Sigma) in 20% Steinberg’s medium. Dimethyl sulfoxide was used as a carrier for the RA. Control embryos were immersed in 1 μl/ml dimethyl sulfoxide (equivalent to the concentration used for RA treatment) in 20% Steinberg’s medium in the absence of RA.

Embryos were dissected in full-strength NAM and the resulting embryos and explants were cultured in NAM/2. RA treatment of explants was carried out as described for embryos above, except that the dishes were coated with 1% agar in NAM.

Whole-Mount in Situ Hybridization

Embryos and explants were fixed for whole-mount in situ hybridization according to Harland (1991) after manual release from their fertilization envelopes. Digoxigenin-labeled antisense RNA probes for whole-mount in situ hybridization were synthesized according to Harland (1991) except that the probes were resuspended directly in hybridization buffer without alkaline hydrolysis. The in situ hybridization protocol of Harland (1991) was used with the following modifications. Tris-buffered saline (50 mM Tris, pH 7.4, 200 mM NaCl) was used throughout rather than phosphate-buffered saline. Embryos were treated with 5 μg/ml proteinase K for 15 min. The 0.2× SSC washes were increased to 1 hr in length, the washes after the antibody incubation were increased in frequency, and the total wash time was lengthened to 7 hr. RNase treatment used 1 μl/ml of an RNase cocktail (Ambion). When the staining intensity was sufficient for viewing, the embryos were passaged through the following washes for 5 min each: 25% methanol, 50% methanol, 75% methanol, 100% methanol, 75% methanol, 50% methanol, 25% methanol. After the washes, the embryos were fixed overnight in Bouin’s fixative to give a yellow counterstain (Harland, 1991). Embryos were cleared and photographed in 2 benzyl benzoate:1 benzyl alcohol.

Sectioning

For serial sectioning, whole-mount stained embryos were rinsed in PBS, dehydrated in ethanol (3×10 min), incubated in xylene (2×10 min), in 1:1 xylene:paraplast (1×30 min), and then overnight in 100% paraplast, and embedded in paraplast. Sections (10 μm) were cut, mounted in Permount, and observed by differential interference optics.

Markers

Complementary DNA clones encoding the following marker sequences were used as probes: neural-specific β-tubulin (Richer et al., 1988), XNkx2.5 (Tonissen et al., 1994), cardiac Troponin I (XTnIc) (Drysdale et al., 1994), cardiac α-actin (Mohun et al., 1984), and X-msr (Dević et al., 1996—individually isolated by M. Saha).

RESULTS

Retinoic Acid Blocks the Expression of Myocardial Differentiation Markers

Treatment of early stage Xenopus embryos with RA causes severe axis defects and general loss of anterior structures (Durston et al., 1989; Sive et al., 1990; Drysdale and Elinson, 1991). In order to focus on heart-specific effects of RA exposure we delayed the time of treatment until the midneurula stage. As shown in Fig. 1A, continuous exposure to 1 μM RA, commencing at the midneurula stage, results in a complete elimination of myocardial differentiation, as assayed using the myocardial marker XTnIc (Drysdale et al., 1994). To confirm that the effect is not restricted to the XTnIc transcript alone, the assay was repeated using probe for cardiac α-actin (Mohun et al., 1984). This marker is expressed in somitic muscle, commencing at stage 14, and also in cardiac muscle, starting at about stage 27 (Hemmati-Brivanlou et al., 1990). As shown in Fig. 1B, no cardiac...
α-actin expression is detectable in the heart region of RA-treated embryos, but expression in the somites appears to be unaffected. In addition to these molecular markers, no beating tissue is detectable when RA-treated embryos are allowed to develop until the beating heart stage (after about stage 34).

RA treatment of *Xenopus* embryos starting at the gastrula stage causes severe reduction or ablation of the heart and most other anterior structures, including anterior neural tissues, cement gland, hatching gland, and eyes (Durston et al., 1989; Sive et al., 1990; Drysdale and Elinson, 1991; Drysdale and Crawford, 1994; Drysdale et al., 1994). In contrast, the later, low-dose treatment described above results in very few defects in superficial morphology. Minor defects, however, are observed in the ventral part of the retina, which does not differentiate normally, in the tail, which does not fully elongate, and in gut morphogenesis. A similar retinal defect has previously been reported in RA-treated zebrafish (Hyatt et al., 1996). To test for defects that might not be visible at the superficial level, neural tissues were detected with probe for neural-specific β-tubulin (Richter et al., 1988). As illustrated in Fig. 1C, neural structures in RA-treated embryos appear normal, with the exception of the trigeminal ganglion which is stunted compared to controls. Note, however, that forebrain structures are present, indicating that no significant reduction of anterior neural structures has occurred. As development proceeds, the appearance of the RA-treated embryos becomes more abnormal and they begin to show severe edema, most likely due to cardiac insufficiency.

Myocardial Development Is Sensitive to Retinoic Acid until the Time of Differentiation

To determine the period during which myocardial differentiation is sensitive to retinoid treatment, embryos were incubated in RA, starting at progressively later times during development. As shown in Fig. 2A, initiation of treatment at any stage up until late neurula (stage 23) completely eliminates all detectable expression of myocardial markers. A significant reduction in the size of the myocardium is observed when treatment is initiated at stage 25 (Fig. 2B). However, when RA treatment is initiated at stage 28, just after the onset of myocardial differentiation (Logan and Mohun, 1993; Drysdale et al., 1994; Cleaver et al., 1996; Hemmati-Brivanlou et al., 1990), there is little or no detectable alteration in heart development (Fig. 2C).

In this study, all embryos were assayed at the same developmental stage (stage 34), regardless of the time at which RA-treatment commenced. It is possible therefore, that the

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**FIG. 1.** RA treatment specifically blocks myocardial differentiation in *Xenopus* embryos. Specific marker sequences have been detected in control (top) and experimental embryos using whole mount in situ hybridization. All embryos were assayed at stage 34. (A) Detection of the myocardial-specific transcript XTnIc. (B) Detection of the α-cardiac actin sequence which is expressed in both cardiac and somitic muscle in the early *Xenopus* embryo. (C) Detection of neural specific β-tubulin type II transcripts.
extent of myocardial marker expression depends on the total time that the developing myocardium is exposed to RA, rather than to the developmental stage at which the treatment is initiated. To test this possibility, embryos were exposed to constant 1 μM RA, starting at stage 28, and allowed to develop for several days. These embryos developed hearts of normal size and morphology, indicating that the stage at which treatment is initiated, rather than the duration, determines the extent of myocardial loss (data not shown).

The Pattern of XNkx-2.5 Expression Is Altered by Retinoic Acid

The homeobox sequence Nkx2.5 (XNkx2.5 in Xenopus) is expressed in cardiac progenitors and the adjacent pharyngeal region in the early embryo (Lints et al., 1993; Tonissen et al., 1994; Schultheiss et al., 1995; Lee et al., 1996). In the Xenopus embryo, XNkx2.5 expression commences during gastrulation (stage 11-12) (Tonissen et al., 1994), the time that the heart is thought to be specified (Sater and Jacobson, 1989; Nascone and Mercola, 1995). Gene ablation studies in mouse indicate that Nkx2.5 is essential for normal cardiac morphogenesis (Lyons et al., 1995) and over-expression experiments in the frog suggest that XNkx2.5 normally functions to maintain precardiac mesoderm in a state competent to respond to additional cardiac differentiation signals (Cleaver et al., 1996). The peak of XNkx2.5 expression in the Xenopus embryo occurs at the tailbud stage (Tonissen et al., 1994; Evans et al., 1995) about 8 hr before the onset of cardiac differentiation. To determine whether XNkx2.5 expression is susceptible to RA, embryos were exposed to continuous RA starting at stage 20/21 and then assayed by in situ hybridization at various later stages of development. It is important to emphasize that stage 20 is well after the onset of XNkx2.5 expression. As shown in Fig. 3A, embryos assayed at stage 24, only about 5 hr after initiation of RA treatment, showed greatly reduced expression of XNkx2.5. This decrease in expression is most pronounced in the precardiac region. In stage 32 embryos, where the heart tube is forming, XNkx2.5 transcripts remain visible in the pharyngeal region, but are undetectable in myocardial cells (Figs. 3B and 3C). As the RA treatment was initiated at the time XNkx2.5 expression is at its peak in the embryo, the rapid decline in XNkx2.5 transcripts suggests that the mRNA has a rather short half-life and that RA treatment effectively blocks expression of the XNkx2.5 gene in cardiac precursor cells. A second tinman-related homeobox gene, cardiac-specific marker sequence, XTnIc, in control (top) and experimental embryos. All embryos are assayed at stage 34. (A) Continuous treatment with 1 μM RA initiated at stage 23. (B) Continuous treatment with 1 μM RA initiated at stage 25. (C) Continuous treatment with 1 μM RA initiated at stage 28.
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XNkx2.3, has an expression pattern almost identical to XNkx2.5 in the Xenopus embryo (Evans et al., 1995; Cleaver et al., 1996) and has the same biological properties when over-expressed in the embryo (Cleaver et al., 1996). In situ hybridization experiments indicate that the profile of XNkx2.3 expression is the same as XNkx2.5 in embryos treated with RA (data not shown).

**Limited Cardiac Morphogenesis Occurs in the Absence of Myocardial-Specific Gene Expression**

To determine the fate of the presumptive myocardial cells in RA-treated embryos, tissue sections were examined after the time of cardiac differentiation. Control and RA-treated embryos assayed for expression of the myocardial-specific marker, XTnIc, are shown in Figs. 4A and 4B, respectively. The control embryo shows a region of intense XTnIc staining, restricted to the myocardial layer. The myocardial tissue has delaminated from the epithelial layer of cells and clearly encloses a region of unstained endocardial tissue. In comparison, the presumptive myocardial layer in the RA-treated embryo shows the same delaminated appearance, but is completely devoid of XTnIc expression.

The presence of endocardial tissue in the hearts of RA-treated embryos was assayed using the endothelial marker sequence, X-msr (Devic et al., 1996). X-msr encodes a G protein-coupled receptor that is related to, but distinct from, the angiotensin II receptor, and which is expressed in developing endothelial tissues including the endocardium (Devic et al., 1996). Control and RA-treated embryos at stage 32 were assayed for X-msr sequences by whole-mount in situ hybridization are shown in Figs. 4C and 4D, respectively. The endocardial layer in the control embryo is visible as a thin tubular structure, continuous with the developing vascular system and the appearance in RA-treated embryos is similar, although the developing vasculature of the head appears somewhat disorganized. Figures 4E and 4F show histological sections through the heart region of control and RA-treated embryos, respectively. The presence of a distinct group of endocardial cells in both control and RA-treated embryos demonstrates that endocardial development is largely resistant to RA treatment that eliminates detectable expression of myocardial markers.

Figure 4G shows the endocardium in sections through a stage 35 control embryo, after the heart tube has folded. The stained endocardial layer is separated from a thin-walled and extensively folded myocardial layer. By comparison, the endocardial layer in the RA-treated embryo (Fig. 4H) is located in close contact to a thick-walled and un-
folded layer of tissue. This tissue layer would normally have formed differentiated myocardium. In the normal heart, the endocardium is separated from the myocardium by a layer of extracellular matrix material (the cardiac jelly) (reviewed in Borg et al., 1995). The immediate juxtaposition of the endocardium and the myocardial layers in the RA-treated embryos suggests that the extracellular matrix has failed to form and that a differentiated myocardium may be necessary for cardiac jelly formation.

Retinoic Acid Can Block Myocardial Differentiation in Explants of Cardiac Progenitors

Although embryos appear relatively normal after RA treatment, some axial defects are observed (Fig. 1). This raises the possibility that RA affects myocardial differentiation by causing defects in other tissues that subsequently result in a block to myocardial differentiation. To test this possibility, the cardiac progenitor region, as deduced by XNkx2.5 expression (Tonissen et al., 1994), was explanted at stage 20/21 and cultured in the presence of 1 μM RA. Control explants normally give rise to a differentiated heart and immediately adjacent tissues (Fig. 5C), but in the presence of RA no cardiac troponin I expression is detected in the explants (Fig. 5D). Since these explants inevitably contain some other tissue in addition to heart, we cannot completely exclude the possibility that RA is blocking myocardial differentiation by altering another tissue; however, our results indicate that the majority of tissues in the embryo are not involved (see Fig. 5B).

DISCUSSION

Retinoic Acid Can Block Myocardial Differentiation in a Stage-Specific Manner

We have demonstrated that exogenous RA can completely block the differentiation of the myocardium when applied at a relatively late stage in the cardiogenic program. The window of RA sensitivity extends until the tailbud stage when myocardial differentiation occurs. Once expression of myocardial differentiation markers has commenced, RA has no discernible effect on the myocardium. Although previous studies have shown that exposure of gastrula stage embryos to RA causes loss of the myocardium (Sive et al., 1990), these embryos suffered severe truncations of all dorso-anterior structures (Durston et al., 1989). Under these conditions, loss of the heart is likely to be a secondary effect, resulting from loss of other dorso-anterior structures that are required to specify the myocardium. This possibility is supported by experiments which demonstrate that a heart-inducing signal emanates from dorso-anterior structures at the time of gastrulation (Sater and Jacobson, 1989; Nascone and Mercola, 1995; Antin et al., 1994).

When embryos are treated with RA during neurula stages (Fig. 1), it is unlikely that the loss of myocardium is a secondary effect, resulting from the influence of RA on another tissue. First, the paucity of general embryonic defects suggests that most tissues are developing normally. Second, no additional signals or trophic factors are necessary for formation of a functional heart in Xenopus after gastrulation (Sater and Jacobson, 1989; Nascone and Mercola, 1995). Third, explants of the presumptive myocardium taken at neurula stages (20/21), and cultured in the presence of RA, fail to form a myocardium (Fig. 5).

Our results strongly suggest that exogenous RA blocks expression of myocardial differentiation markers in general, and not just the expression of a subset of myocardial genes. This is most clearly demonstrated by expression of the cardiac α-actin gene, which is completely absent in the cardiac region of the embryo, but remains at normal levels in the somites (Fig. 1). In addition, late stage embryos fail to exhibit any detectable heart beat, indicating a failure to form the contractile apparatus. On the other hand, expression of endocardial genes and formation of the simple heart tube appears to proceed normally in the presence of RA. The fusion of the two heart primordia has already been initiated at the time RA is added (Nieuwkoop and Faber, 1994), and, at later stages, a morphologically normal tube is present at the position of the myocardium (Figs. 4G and 4H). Overall, these observations suggest that the primary defect lies in the differentiation of the myocardium and does not result
FIG. 5. RA treatment of heart region explants. Explants of the presumptive cardiac region were taken from normal embryos at stage 20/21 and cultured until stage 33/34, well after the time of cardiac differentiation. In all panels, differentiated myocardial tissue is detected by whole-mount in situ hybridization to XTnIc transcripts. The fate of the explanted region can be seen by comparing a normal embryo (A), to a similar embryo from which the presumptive cardiac region has been removed (B). Control explants differentiate to form cardiac tissue (C), while explants incubated in 1 μM RA exhibit no detectable cardiac troponin I expression (D).

from the elimination of presumptive myocardial cells. We are not aware of any other example where limited morphogenesis occurs in the absence of overt differentiation.

How Is Retinoic Acid Exerting Its Effect?

It is clear that retinoids play a role in normal heart development. Mice lacking function of specific retinoid receptors, or combinations of these receptors, often have defects in cardiac development (Sucov et al., 1994; Kastner et al., 1995) although in all cases myocardial differentiation markers are expressed. It should be noted that a possible explanation of the RXRα knockout phenotype is that RXRα may normally act to block precocious differentiation of the myocardium (Kastner et al., 1995), raising the possibility that exogenous RA may be acting on such a function. Blocking retinoid signaling by dietary insufficiency of vitamin A or by addition of anti-RA antibodies also results in cardiac defects (Twal et al., 1995). Although the mechanism remains unknown, addition of excess RA often results in similar defects to those observed when RA signaling is disrupted (Means and Gudas, 1995). While addition of exogenous RA is known to influence heart development in other species (Osmond et al., 1991; Stainier and Fishman, 1992; Yutzey et al., 1994), the observed effects do not include loss of myocardial gene expression. The increased sensitivity of the developing Xenopus myocardium may result from a
greater effective dose of RA, because the embryos are immersed in a solution of RA. This is consistent with the observation that neural defects resulting from RA exposure are also more pronounced in Xenopus (Durston et al., 1989) when compared to mammalian embryos (Morriss-Kay et al., 1991; Conlon and Rossant, 1992).

The RA signaling pathway is capable of regulating gene expression (Giguere, 1994) and it is possible that exogenous RA alters the expression of a gene required for myocardial differentiation. One potential target of RA regulation is the homeodomain gene Nkx2.5 (Lints et al., 1993; Komura and Izumo, 1993; Tonissen et al., 1994), which is expressed at high levels in the developing heart and which is required for correct heart development (Lyons et al., 1995). We have shown that RA treatment rapidly reduces levels of XNkx2.5 transcript in the presumptive cardiac region (Fig. 3). When Nkx2.5 expression is eliminated in the mouse, a beating heart still forms and most cardiac-specific gene expression is maintained, but the resulting heart is poorly developed (Lyons et al., 1995). Therefore, elimination of Nkx2.5 expression by RA would not be expected to completely block myocardial differentiation. However, it is now known that multiple tinman-related sequences are expressed during heart development in frog, zebrafish, and chicken (Evans et al., 1995; Lee et al., 1996; Buchberger et al., 1996), raising the possibility that additional tinman-related sequences are expressed during heart development in mouse and these may act to lessen the impact of Nkx2.5 elimination. We have observed that XNkx2.3 expression is also eliminated in the heart region by RA treatment of frog embryos. Thus RA is either responsible for blocking transcription of both tinman-related sequences or is acting on a common regulatory pathway.

Retinoic Acid Treatment Mimics the Process of Heart Field Reduction

In both Xenopus and zebrafish, the heart field is delineated by embryological experimentation, and it appears to correlate with the early expression domains of tinman-related genes (Sater and Jacobson, 1990; Stainier et al., 1993; Tonissen et al., 1994; Chen and Fishman, 1996). Diminution of the heart field during development appears to correspond to a reduction in the region expressing the tinman-related sequences. On the other hand, increasing the level of expression of tinman-related sequences enlarges the cardiac region (Cleaver et al., 1996; Chen and Fishman, 1996). The ability of retinoic acid to block cardiac differentiation has at least two features that resemble this process. The time at which differentiation of the myocardial precursors can be blocked corresponds to the time at which heart field contraction occurs in the embryo (Sater and Jacobson, 1990). When the heart begins to differentiate, there is no longer any reduction in the heart field and RA has no effect on the heart. In addition, the reduction in the heart field appears to correspond to a reduction in the XNkx2.5/XNkx2.3 expression domain and we have found that the level of both XNkx2.5 and XNkx2.3 transcripts decline rapidly after RA treatment. However, it is unlikely that RA is the normal mediator of heart field contraction. While targeted disruption of retinoic acid receptors has a marked effect on cardiac development, enlarged hearts are never observed (Sucov et al., 1994; Kastner et al., 1995). An increase in heart size might be expected if retinoic acid is normally responsible for reduction in the heart field. It is plausible, however, that understanding the mechanism by which RA has its striking effect on myocardial differentiation, will provide clues to the normal processes underlying heart field contraction.

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REFERENCES


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