Function of Vitamin A in Vertebrate Embryonic Development1,2

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ABSTRACT Advances in molecular biology and retinoic acid receptor research have significantly contributed to the understanding of the role of vitamin A during vertebrate development. Examination of the function of this vitamin during very early developmental stages using the completely vitamin A–depleted avian embryo has revealed that the vitamin A requirement begins at the time of formation of the primitive heart, circulation and specification of hind-brain. The lack of vitamin A at this critical time results in gross abnormalities and early embryonic death. In rodent models, vitamin A deficiency can be targeted to later gestational windows and documents the need for vitamin A for more advanced stages of development. Major target issues of vitamin A deficiency include the heart, central nervous system and structures derived from it, the circulatory, urogenital and respiratory systems, and the development of skull, skeleton and limbs. These abnormalities are also evident in mice mutants from retinoid receptor knockouts; they have revealed both morphological and molecular aspects of vitamin A function during development. Retinoic acid receptors (RAR) in partnership with retinoid X receptor (RXR) appear to be the important retinoid receptor transcription factors regulating vitamin A function at the gene level during development via the physiologic ligand all-trans-retinoic acid. Homeostasis of retinoic acid is maintained by developmentally regulated vitamin A metabolism enzyme systems. Inadequate vitamin A nutrition during early pregnancy may account for some pediatric congenital abnormalities. J. Nutr. 131: 705–708, 2001.

KEY WORDS: • vitamin A • avian development • retinoic acid

The essentiality of vitamin A nutrition throughout the life cycle has been well established. Most importantly, the requirement for vitamin A begins with embryonic life as noted by early nutritionists who reported that maternal insufficiency of vitamin A during pregnancy results in fetal death or congenital abnormalities in the offspring (1–4). During the last decade, there has been great interest in the area of vitamin A function in development as reflected in a number of recent detailed reviews on the subject (4–8).

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An important approach to the examination of molecular mechanisms of retinoid action in developmental regulation is the use of in vivo embryo model systems in which the function of vitamin A has been diminished or completely eliminated by removing the vitamin. The absolute essentiality of vitamin A for embryogenesis is most clearly demonstrated in the VAD avian embryo, i.e., the quail embryo retinoid ligand knockouts. The completely VAD embryos develop gross abnormalities in the cardiovascular and central nervous systems and trunk and die by day 4 of embryonic life (3,4,6,7,21–23). Importantly, the VAD embryo can be “rescued” and normal development restored by administration of the physiologic ligand for RAR, all-trans-retinoic acid, or its precursor, retinol. Bioactive retinoids must be administered to these embryos during early development so as to be present during the critical window of time in which important developmental events are specified, i.e., the formation of heart, cardiovascular system, hindbrain, foregut and probably other events (21,23). With this model, it is possible to examine morphological, anatomical and molecular biology aspects during development that are solely attributable to vitamin A. The ability to rescue the VAD embryo at a precise time during development makes the avian retinoid ligand knockout model a powerful tool for the elucidation of vitamin A function during early development.

Using rats as a mammalian model, it is possible to obtain near vitamin A deficiency in the dams and to target embryonal vitamin A insufficiency to distinct gestational windows (3,4,6,7,24–26). These rat embryos exhibit specific cardiac, limb, ocular and central nervous system (CNS) abnormalities, some of which have certain features similar to those reported in retinoid receptor knockout mice (25). Abnormalities in hindbrain development of the rat embryos (26) are similar to those reported in VAD quail embryos (7,27) and suggest that even partial vitamin A deficiency affects the sensitive developing CNS. These studies have also revealed the importance of vitamin A in fetal lung and kidney development (24,28,29).

Another in vivo approach to eliminate vitamin A–active forms is to block RA with an anti-RA antibody (30) or to inactivate the RA generation pathways (4,7). Knockout mice embryos of the RA synthesizing enzyme RALDH2 (11) have many abnormalities similar to those of the VAD quail embryo, but complete vitamin A deficiency was not obtained, probably due to the presence of other RA-generating systems. Partial depletion of RA may also be achieved by an overexpression of CYP26, an enzyme that degrades RA (4,13).

**Heart and Blood Vessel Development.** A great deal of information on the effects of retinoids on heart development has been gathered from ectopic application of excess retinoids to embryos (6,31,32) but physiologically more relevant are the experimental approaches that diminish or eliminate vitamin A function. Maternal insufficiency of vitamin A during pregnancy results in fetal death or severe abnormalities in the offspring, including abnormal heart development; many of the heart defects have been recapitulated in fetuses generated from various combinations of retinoid receptor knockouts. Heart abnormalities obtained include a thin-walled dilated heart cavity, abnormalities in ventricular chambers and defects in the outflow tract (3,4,6,18–20,22,25,31). Important information has also been obtained by molecular analysis (9,18–20), but the primary retinoid targets have not been identified. The phenotypes obtained may reflect secondary lesions and abnormalities due to RXR functions with other nuclear receptors (4,9). In contrast, the quail embryo retinoid ligand knockout model allows an analysis of developmental regulation solely attributable to vitamin A and reveals the full function of this vitamin. The model has an advantage for studies of vitamin A function during early precardiac and subsequent heart-forming stages by “rescue” manipulations to restore normal gene expression and cardiogenesis. The VAD cardiac phenotype in this avian model is highly reproducible and, together with molecular analysis, allows us to draw certain conclusions about the initial cardiogenic events regulated by vitamin A (3,6,21–23). The developing heart is a grossly abnormal, thin-walled, dilated and distended structure, without chambers, but it contracts until the embryo dies. This is not surprising because the expression of early cardiogenic genes that regulate heart precursor cell differentiation into cardiomyocytes is not affected by the lack of vitamin A (22). In contrast, exogenous treatments with RA induce differentiation of precursor cells into cardiomyocytes (31).

The concept of a specific retinoid function in axial specification has a long history, originating with the identification of RAR responsive elements in some of the evolutionarily conserved axial patterning genes (33). It has been perpetuated by results from numerous studies in which exogenous RA applied to embryos of various species at various stages of development affects the specification of body axes, heart asymmetry and limb patterning (5,8,31–33). These studies suggested a specific role for RA in heart asymmetry determination (23,31,32). However, recent evidence points to vitamin A having a general rather than a specific role, i.e., it appears that vitamin A is required to provide a proper environment for the expression of adequate levels of heart asymmetry genes (23).

This concept is supported by evidence from the literature that the generation and distribution of RA in the embryo as well as the expression patterns of vitamin A metabolism enzymes and retinoid receptors are symmetric (7,11–14,21).

A major developmental defect that may be linked directly to the early embryolethality of the VAD quail embryo is the absence of a cardiac inflow tract (3,6,22), i.e., the VAD heart has no opening at its caudal end where the extraembryonic blood vessels converge into vitelline veins to deliver blood to the embryonic heart for distribution to the embryo. This defect has not been reported in any of the mammalian in vivo models addressing vitamin A function during embryogenesis. The formation of the cardiac inflow tract may be linked to the expression of the retinoid-regulated cardiac transcription factor GATA-4 in the posterior heart-forming area where this gene is involved in a BMP2 pathway that specifies the endodermal structures of the heart and the underlying foregut primordia, and where apoptosis is observed in the VAD embryo (34). In these embryos, the extraembryonal vascular networks are sparse and fail to converge at the level of the cardiac inflow tract (35; Fig. 1). Defects in vitelline vessel formation have also been observed in cultured mouse embryos when the transfer of retinol from the yolk-sac to the embryo is prevented (36). Anomalies in vasculogenesis have not been reported in retinoid receptor knockout mice, but may have contributed to embryolethality.

**The Central Nervous System.** Vitamin A plays an important role in development of the central nervous system (CNS) (4,7,10,14,26,27,37). Rescue studies and functional analysis of VAD quail embryos have revealed that vitamin A is required at the time of specification of the posterior hindbrain, for the subsequent specification of segments in that region and for neurite outgrowth and neural crest survival (7,27). Studies with in vivo vitamin A–deprived rodent embryos (14,26,38) revealed less severe CNS defects, suggesting that the deficiency was not complete. When such embryos survive to more advanced developmental stages, abnormalities are seen in the developing visual system and the retina, in inner ear primordia and in craniofacial and spinal cord development (4). Retinoid
gene, an enzyme involved in the generation of RA, do not appear to have limb buds (11), suggesting species differences.

Using partially vitamin A–depleted rodent models in which the fetuses survive longer and later gestational windows can be examined, it was demonstrated that vitamin A is specifically required during midgestation for fetal lung development and neonatal survival (24,42). Abnormal cell differentiation and diminished expression of elastin gene and a growth-arrest gene were associated with abnormal lung morphology (42), also seen in mice with deletions in RAR genes (29). Compound mutants of RAR also exhibit congenital respiratory tract abnormalities (4,19). The importance of adequate maternal/fetal vitamin A nutrition in prevention of neonatal lung injury has been demonstrated clinically and in animal models (28). Similarly, renal development is affected by retinoids (43), also noted in the RALDH2 knockout mice (11); congenital malformations in the urogenital system are seen in fetuses of RAR compound mutant mice (4).

Summary and Perspectives. Early animal studies on vitamin A requirement during pregnancy as well as more recent rodent depletion models have provided important information regarding the role of this vitamin during specific gestational windows. However, complete vitamin A deficiency is necessary to reveal the full function of vitamin A during early development as best illustrated in the avian embryo vitamin A–deficient (VAD) quail embryo. Knockout model. Results from these experiments suggest that the requirement for vitamin A activity in the embryo begins at the time of first organ system initiation, not earlier. This hypothesis is in agreement with the conclusions reached by Durston et al (33) who noted that the gain and loss of function experiments with retinoid receptors do not demonstrate significant changes during early embryogenesis and thus challenge the existing hypothesis that RA signaling is essential at the time of embryonic cell organization and initial axial patterning. Results from the avian studies provide strong evidence that the absolute vitamin A requirement begins at the time of formation of the primordial heart tube, the linking of the extraembryonal vascular system to the developing heart and the specification of hindbrain. These critical events in the avian embryo take place at the same developmental time and correlate to first 2–3 wk of human pregnancy, emphasizing the importance of adequate vitamin A nutrition during initial stages of pregnancy. If vitamin A is completely lacking during this critical time, development takes place along a default pathway, resulting in gross abnormalities and early embryolethality. In marginally vitamin A–deprived rat embryos or with some retinoid receptor knockouts, the requirement for vitamin A can be demonstrated for later, more advanced developmental events. Certain retinoid receptor knockouts recapitulate many of the vitamin A deficiency symptoms, but do not provide a clear vitamin A deficiency phenotype because of receptor redundancy and the pleiotropic effects from RXR acting as partners with multiple nuclear receptors (4,9,18,31).

Altogether, all observations support the requirement for vitamin A during multiple stages of development for numerous tissues and organs. Consistent with the many roles of vitamin A in development, retinoid receptors are widely expressed in the vertebrate embryo (4,12,31). Retinoid receptor knockout studies have revealed that for developmental function, there is very little redundancy among the RAR subtypes, and that RXRs is the major partner for the RAR in RA signaling (4). The RAR-RXR heterodimer is most likely activated by the RAR ligand, all-trans-retinoic acid, with RXR acting as enhancers in RA signal transduction (9). The present data suggest that during embryogenesis, all-trans-retinoic acid is the...
solute active form for all retinoid functions involving retinoid receptors. Vitamin A function during embryogenesis is locally regulated by a spatiotemporal developmental expression of RA-generating and -inactivating enzymes and further fine-tuned through a diversity of receptors and their isoform combinations. A number of genes are known to have RAR responsive elements (4,8,10), but it is not known which genes are causally and directly linked to the VAD phenotype. At this time, no immediate RA target genes have been identified for any developmental event, and no single protein, other than the RAR and RXR proteins, is known to be linked directly to vitamin A function. RA effector genes may be important genes involved in mainstream functions such as cell division, differentiation and energy metabolism, biological processes that have long been recognized as hallmarks of vitamin A action.

Understanding the function of vitamin A nutrition during development is of great clinical importance because population surveys estimate that ~3% of all children born in the United States have heart malformations at birth (44) with 70% of these of unknown etiology, including congenital heart disease, the most prevalent human birth defect (45). Some of the heart abnormalities obtained with retinoid receptor knockouts or resulting from insufficient vitamin A resemble the most common heart defects in humans (45).

LITERATURE CITED


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