Inhibition of cranial neural crest cell development by vitamin A in the cultured chick embryo

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SUMMARY

Chick embryos at stage 8, prior to neural crest cell migration, were explanted on whole egg medium with or without vitamin A and cultured for 3 days. Sections through the head regions showed that the cranial neural crest cells had migrated into the first visceral arch in the controls but were absent from this structure in the treated embryos. These observations suggest that vitamin A inhibits neural crest cell development or migration, an effect which may in part account for the facial malformations produced by excess vitamin A.

INTRODUCTION

Vitamin A causes a variety of facial and limb malformations in rodents, depending upon the gestational age at which it is administered. Morriss (1973) has shown that a single dose of retinol palmitate given to the rat on day 8, 9, or 10 produces mainly facial malformations while administration on day 11 or 12 results predominantly in defects of the limbs.

[³H]Thymidine cell marking procedures used for the study of chick embryos have shown that most of the facial mesenchyme is derived from the neural crest cells (Johnston, 1966). In the rat, where early facial development is basically similar to that of the chick, the neural crest cells migrate from the neural plate and into the facial region during days 9 and 10 (Johnston & Hassell, unpublished observations). This period of greatest susceptibility to vitamin A-induced teratogenicity with regard to facial malformations appears to coincide with the migration of the neural crest cells. These observations suggest that vitamin A may alter neural crest cell development. Similar hypotheses has been put forward by Morriss (1975) and Poswillo (1975).

The explanted chick embryo system (Spratt, 1947; Klein, McConnell & Requier, 1964) permits the use of precisely staged embryos which can be

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exposed to a variety of agents during a 3-day culture period. Klein et al. (1964) has shown that compared to in ovo development, in vitro organ development proceeds normally but growth is slower. This system was employed in this study to examine the effect of vitamin A on cranial neural crest cell development.

MATERIALS AND METHODS

Fertile eggs from White Leghorn hens were obtained from Truslow Farms (Chestertown, Md.) and incubated for approximately 30 h at 37.5 °C prior to explantation. The embryos were then dissected and cultured according to the technique of Spratt (1947) as modified by Klein et al. (1964). Embryos with extra-embryonic membranes were cut free of the yolk, and those embryos of stage 8 (Hamburger & Hamilton, 1951; 5 or 6 pairs of somites) were used for culture. After removal of the overlying vitelline membrane, the extra-embryonic membrane peripheral to the sinus terminalis was trimmed away. The explants were placed ventral surface down on a semi-solid nutrient medium and cultured at 37.5 °C in an atmosphere 25% O₂ + 75% air for the first 24 h and in 100% O₂ for the next 48 h. At the end of the 72 h culture period the embryos were fixed in 2.5% glutaraldehyde (LADD) in 0.1 M cacodylate buffer, pH 7.4. The embryos were embedded in paraffin, cut at 8 μm and stained with hematoxylin and eosin.

The culture medium used was the whole egg medium of Britt & Herrmann (1959) in which the white and yolk of a fresh egg were combined with an equal volume of a 1.5% agar solution in chick Ringer's at 50 °C. Retinol (Sigma, final concentration 10, 50, 100 μg/ml of medium) was dissolved in 95% ethanol and mixed with the culture medium before it was dispensed in 1 ml aliquots and allowed to solidify.

RESULTS

Figure 1 shows a section through the head of a control embryo cultured for 72 h and which has reached stage 20. By this stage, the neural crest cells (NC) have migrated from the neural folds and appear in the first visceral arch as loosely packed cells surrounding a densely packed core of mesodermal cells (M). The arrows indicate the path and direction of neural crest cell migration. Fig. 2 shows a cross-section through the head of an embryo cultured in the presence of 50 μg of retinol/ml medium for 72 h. Although the densely packed mesodermal core of cells is present in the first aortic arch, the number of neural crest cells surrounding the mesodermal core is greatly reduced. Furthermore, there are a number of other differences: the epithelial lining of the foregut is thickened, the cavity of the foregut is distorted and enlarged, the dorsal aortas are enlarged and there appears to be an increased cell density in the region (circled areas) near the base of the visceral arch. Similar results were obtained at 100 μg retinol/ml, but embryos cultured on medium containing 10 μg/ml were unaffected.
Fig. 1. Cross-section through the head region of a control embryo stage 20 cultured for 72 h. Neural crest cells (NC) have migrated into the first visceral arch. The arrows indicate the path and direction of neural crest cell migration. NT, neural tube; DA, dorsal aorta; FG, foregut; NC, neural crest cells; M, mesodermal cells; N, notochord. x 130.

Fig. 2. Cross-section through the head region of an embryo cultured for 72 h on medium containing 50 μg retinol/ml. The number of neural crest cells in the first visceral arch is greatly reduced and an increased cell density (circled areas) is observed near the base of the visceral arch. NT, neural tube; DA, dorsal aorta; FG, foregut; M, mesodermal cells; N, notochord. x 130.

DISCUSSION

The results of this study show that vitamin A prevents the appearance of the cranial neural crest cells in the first aortic arch of cultured chick embryos. This particular arch gives rise to the lower jaw, a structure which was shown to develop abnormally in rats treated with vitamin A at a comparable developmental age (Morriss, 1973). It may be that altered neural crest cell development accounts for the facial malformations produced by vitamin A.

Normally, the neural crest cells migrate out from the neural tube towards the aortic arches in a hyaluronate-rich cell-free space between the mesoderm and the ectoderm (Johnston, 1966; Pratt, Larson & Johnston, 1975). Although the fate of the neural crest cells in the treated embryos cannot be conclusively determined
from the data presented, the increased cell density observed near the base of the visceral arch may actually represent some of the crest cells or their remnants that failed to reach the first visceral arch. In contrast, the core of condensed mesodermal cells in the first arch does not appear to be affected. These observations suggest that vitamin A may, in part, alter the migration of the crest cells. In support of this suggestion, Kwasigroch & Kochhar (1975) have also reported that vitamin A impairs mesenchymal cell movement in cell culture. However, one cannot rule out the possibility that vitamin A inhibits neural crest cell proliferation or causes cell death, which could also account for the reduced numbers of neural crest cells seen in the first aortic arch.

Vitamin A has been shown to alter the biochemical and morphological properties of cultured epidermal cells (Yuspa & Harris, 1974; DeLuca & Yuspa, 1974), cartilage cells (Fell & Dingle, 1963), and 3T3 cells (Kochhar, Dingle & Lucy, 1968) at concentrations of 3–12 μg/ml, which were lower than the effective concentrations (50–100 μg/ml) required in the present study. However, in the experiments reported here, the explants were cultured on top of the solidified medium rather than submerged and so must rely on the extra-embryonic membrane for metabolism and transfer of components of the medium to the embryo (Hassell & Klein, 1971).

Although the biochemical mechanisms by which vitamin A causes teratological effects has not yet been determined, there are several possibilities. First, Fell & Dingle (1963) have shown a reduction of matrix staining in cartilage cells treated with vitamin A and have suggested that vitamin A causes a destabilization of lysosomal membranes, leading to the release of enzymes that degrade the matrix. In the case of the neural crest cells, released lysosomal enzymes may alter the hyaluronate-rich matrix through which they migrate. However, more recent evidence suggests that vitamin A may also alter synthesis of glycosaminoglycans or glycoproteins. Yuspa & Harris (1974) and DeLuca & Yuspa (1974) have shown that vitamin A causes an increase in glycoprotein synthesis or glycosylation and PAS staining in epidermal cells. Kochhar et al., (1968), using 3T3 cells, showed that vitamin A increased the secretion of a glucosamine-labeled product into the medium. Since it has been shown the neural crest cells synthesize glycoproteins (Greenberg & Pratt, 1977) and migrate through a hyaluronate-rich matrix (Pratt et al., 1975) synthesized at the time of migration, it may be that vitamin A alters cell surface glycoproteins or the secretion and distribution of the matrix in which the neural crest cells migrate.

REFERENCES

Vitamin A and neural crest cell development in chick embryos


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