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Accumulation of Retinoids in Embryonic Neural and Neural Crest Cells as Part of the Mechanism of Teratogenesis

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INTRODUCTION

Even though drugs for a long time have been tested with respect to teratogenic effects, there are very few data on the dose-effect relationship for the embryo. This contribution will focus on the distribution of retinoic acid in the early rodent embryo, and relate this distribution to known effects on development.

Vitamin A is since long known to be a teratogen (3). Megadoses of vitamin A have been suspected, but not proven, teratogenic in man. The last few years, retinoids have been introduced as pharmaceuticals to be taken orally, either as anti-acne drugs (Accutane, 13-cis-retinoic acid), or against psoriasis (Etretinate, an ester of a retinoic acid derivative). Presently (mid 1986), 50-100 cases of more or less severe malformations have been reported (for review, see ref. 7). As is the case with experimental animals exposed in the earliest stages of gestation, also children born to treated mothers exhibit mainly microtia, brain, eye, and facial malformations, in addition to thymus and heart anomalies. The good correlation between clinical and animal experimental findings (7) makes the experimental animals suitable to work with from a mechanistic point of view.

RESULTS AND DISCUSSION

When All-trans-retinoic acid-15-^{14}C (RA-^{14}C) was administered to pregnant mice on Day 9 (8 1/2 days) of gestation, and the mice were used for autoradiography studies (10, 11), embryonic radioactivity was observed preferentially in the neuroepithelium of the neural folds in the head region at one and 4 h (not shown). Already at this early developmental stage, but also later, the concentration in the neuroepithelium along the neural tube varied considerably. Thus, at Day 9 through Day 12, segments of the central nervous system had high radioactivity, up to 10 times higher than maternal blood, while others had low activity (Fig. 1). From Day 13 and onwards, a considerably lower uptake of RA-^{14}C in the central nervous system was observed.

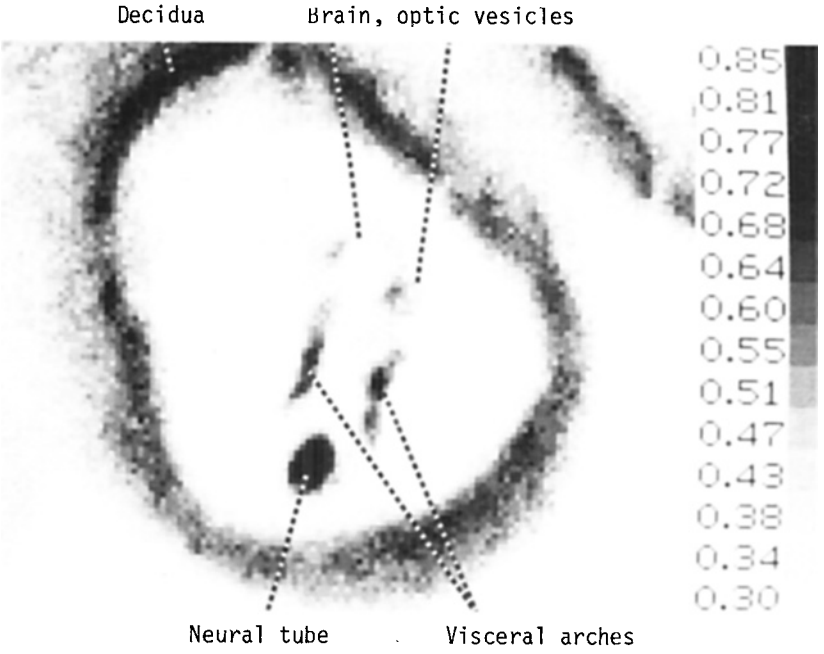

Fig. 1. Image of autoradiogram (Day 10, 4 h after RA-^{14}C injection) produced by computerized densitometry (see article by R. d'Argy, this issue). It is part of the uterus, where the decidua has a relatively high concentration (dark encircling of the embryo). The highest concentration in embryo is found in the neural tube and the visceral arches.

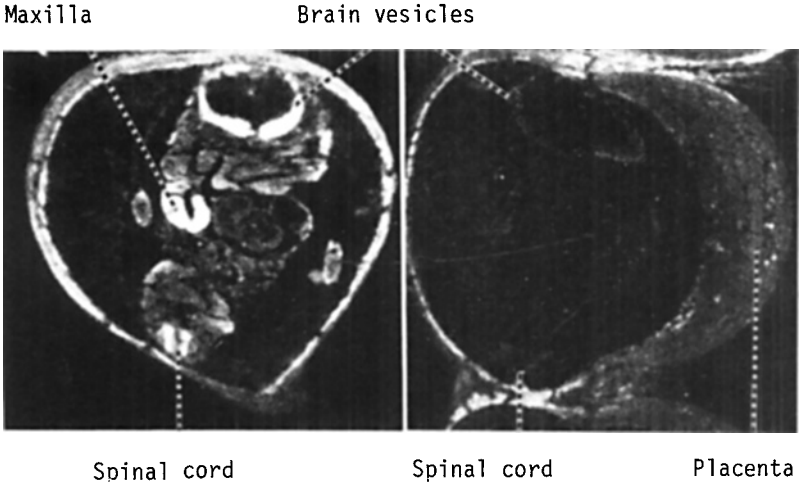

Fig. 2. Autoradiograms showing parts of uteri from two mice at Day 11 of gestation, injected i.v. with RA-^{14}C (1 mg/kg b.w.) and killed 4 h later. The autoradiogram to the left is from a control (vehicle-injected) mouse, the one to the right is from a mouse injected with 50 mg/kg b.w. of nonlabelled RA 1 hr before the radioactive dose. Note the very marked reduction in embryonic radioactivity, which was most pronounced in organs with a normally high activity (e.g. maxilla, brain vesicles, spinal cord).

Not only the neuroepithelium, but also a cluster of cells in close apposition to the edge of the neural fold, were found to accumulate activity after RA-^{14}C injection at Day 9. The same high activity was observed at Day 10, now in the area of the visceral arches, and at Days 11-12 in the maxillary region.

It is striking that the cells outside the neural tissues, where activity has been observed, have a position in the embryo that very much resembles what has been described for cranial neural crest cells (4). It has been shown that cranial neural crest cell migration in early embryogenesis may be disorganized by RA, and that these cells can be damaged or killed, while no other embryonic cells are visibly compromised (12). RA dramatically reduces the size of the first and second visceral arches (where we found RA to accumulate), which eventually give rise to the maxilla, mandible, and ear (1).

Since the activity observed here was 1) restricted to certain cell types, and 2) varied in concentration within one and the same tissue (the neuroepithelium), it was suggested that the uptake may be mediated by some binding protein or other accumulatory mechanism. If so, it might also be saturable. In experiments where relatively low i.v. doses of RA-^{14}C (1 mg/kg b.w.) were combined with i.p. injections of high doses of nonlabelled RA (50 mg/kg b.w.), the normally very specific uptake of radiolabel in the embryonic target organs was inhibited, although maternal blood and placental concentrations were unaffected (Fig. 2). In this situation, most embryonic organ anlagen were at the level of maternal blood concentration.

CONCLUSIONS

It remains to be shown what is the mechanism of RA accumulation in the neural and presumed cranial neural crest cells. However, we suggest an interpretation based on one of the current theories of RA (and vitamin A) action via specific cytoplasmatic receptors. RA receptors have been found in many malignant cells, including neural tumours (neuroblastomas) (2) and are more abundant in organs of young than in older animals (6). RA is known to influence genomic expression (5) and differentiation (8), which suggests an interaction with the cell nucleus.

It is tempting to assign a role for the putative receptor in embryonic development. Both excess and deficiency of vitamin A causes developmental errors (3, 13). It is interesting to note that RA administered to vitamin-A-deficient animals can prevent malformations and embryonic death and resorptions, throughout the organogenetic period, but not later, in the rat (9). It may thus be that a RA receptor is expressed most abundantly in early gestation and that it is RA (or some metabolite of RA) rather than vitamin A which is necessary for early embryonic development. Too high doses may however cause an "overstimulation" of RA-dependent events, leading to CNS, eye, ear, facial, thymus, and great vessle malformations.

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