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Genesis of Teratogen-induced Holoprosencephaly in Mice

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Abstract

Evidence from mechanical, teratological, and genetic experimentation demonstrates that holoprosencephaly (HPE) typically results from insult prior to the time that neural tube closure is completed and occurs as a consequence of direct or indirect insult to the rostral prechordal cells that induce the forebrain or insult to the median forebrain tissue, itself. Here, we provide an overview of normal embryonic morphogenesis during the critical window for HPE induction, focusing on the morphology and positional relationship of the developing brain and subjacent prechordal plate and prechordal mesoderm cell populations. Subsequent morphogenesis of the HPE spectrum is then examined in selected teratogenesis mouse models. The temporal profile of *Sonic Hedgehog* expression in rostral embryonic cell populations and evidence for direct or indirect perturbation of the Hedgehog pathway by teratogenic agents in the genesis of HPE is highlighted. Emerging opportunities based on recent insights and new techniques to further characterize the mechanisms and pathogenesis of HPE are discussed.

Keywords

holoprosencephaly; prechordal plate; teratogenesis; mouse

Introduction

While the genesis of holoprosencephaly (HPE) has been studied in a number of mammalian and non-mammalian species (e.g. amphibians [Holtfreter and Hamburger, 1955], fish [Blader and Strahle, 1998], chick [Cordero et al., 2004], rat, sheep [Keeler, 1975]), recent investigations employing mice have been particularly informative. This is true for both genetic and environmental etiologies, the former of which has recently been reviewed by Schacter and Krauss [2008], and Geng and Oliver [2009]. Herein, the focus is on the development of teratogen-induced HPE in mice.

As opposed to most gene manipulation-based investigations, teratogenesis studies afford the opportunity to examine the result of insult occurring within a narrow window of development. In mice, the critical period for the induction of HPE is between gestational days (GD) 7 - 8.5; i.e. during gastrulation and neurulation stages. This corresponds to human development between the middle of the 3rd week to early in the 4th week post-fertilization. At these early embryonic stages, humans and mice are remarkably similar and interspecies extrapolations regarding molecular and cellular mechanisms appear well founded.

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It has long been recognized that the cell populations that are initially affected to result in the median forebrain defects characteristic of HPE are those that normally lie rostral to the notochord; i.e., they are prechordal and include those endodermal and mesodermal cells that are subjacent to the rostral-most aspect of the neural plate and/or the neuroepithelial cells of the prospective forebrain, itself. As early as the 1930s, Adelman [1934] reported cyclopia resulting from mechanical removal of the prechordal mesoderm in amphibian embryos. Holtfreter and Hamburger, in [1955], showed that this same endpoint could result from teratogen-mediated interference with gastrulation, the developmental process that results in formation of the mesoderm and definitive endoderm of the embryo. More recently, the molecular signaling events required for the development of the prechordal tissues, including those associated with forebrain induction and subsequent early forebrain development have been elucidated [Gunhaga et al., 2000; Geng et al., 2008; Aoto et al., 2009; Maurus and Harris, 2009]. Comparable to insult by genetic manipulation, a variety of teratogens that cause HPE (though probably not all) appear to directly or indirectly affect hedgehog (Hh) signal transduction, involving either Hh generating or receiving cell populations.

Normal Development during the Critical Period for HPE Induction

Figures 1-4, which are comprised of light and scanning electron micrographs, are designed to provide a framework for understanding developmental events/tissues that, when disrupted, can result in HPE. A majority of the images are of mouse embryos, but a few micrographs of chicken embryos are included to provide additional pertinent information. Focus is on the cellular relationships and morphology of prechordal cell populations; i.e. cells that are located rostral to the notochord. In a recent review, Müller and O'Rahilly [2003] point out difficulties/discrepancies in the terminology that is used to describe the cell populations that are subjacent to the developing rostral neural plate. These authors suggest abandoning the commonly (and, in many cases, interchangeably) -used term, "prochordal plate" in favor of the term "prechordal plate". They define the prechordal plate as being a temporary cellular plaque that is situated rostral to the notochordal process at Carnegie stage 7 & 8 in the human (days 16-18 post-fertilization) and that comes to underlie the rostral part of the neural plate by Carnegie stage 9 (20-21 days; the time when the first pairs of somites are visible). While some confusion surrounds the origin of the prechordal plate cells, they are generally described as arising from the epiblast and as being endodermal. This is in contrast to the notochord, which at early stages of development is comprised of a cell layer that is laterally continuous with the endoderm, but is considered mesodermal [Sulik et al., 1994]. As illustrated in Figure 1, which shows Hamburger and Hamilton (H&H) Stage 7 and 8 chick embryos (stages when 1-4 somite pairs are present), the rostral-most tissue underlying the forebrain, i.e. the prechordal plate, is composed of an organized tall columnar cell population that is continuous with the foregut endoderm. Between the prechordal plate and the developing notochord is a mesenchymal (fibroblastic/non-epithelial) axial population of cells that is considered prechordal mesoderm. These prechordal mesoderm cells are located subjacent to the developing midbrain, while a more condensed axial population that forms the notochord is subjacent to the hindbrain (Fig. 1b). Studies employing chicks have shown that just prior to the stage shown in Figure 1, expression of molecular markers characteristic of ventral telencephalic cells are specified in response to Shh signals derived from the region of the node and (possibly) the prechordal plate region [Gunhaga et al., 2000].

Regarding development of prechordal and notochordal cell populations in mice, Sulik et al, [1994] showed that on the ventral surface of GD 7 embryos (Theiler Stage 10, corresponding to approximately 16-18 days post-fertilization in humans, when gastrulation is initiated and the first mesodermal cells can be identified), the anterior midline is identifiable by the presence of small clusters of mono-ciliated cells (Fig. 2 a, b). As

development progresses, a larger grouping of these cells are present near the apex of the cup-shaped embryo and comprise the notochordal plate. The notochordal plate extends rostrally from the node (Fig. 2 c, d), the latter of which is at the anterior-most aspect of the primitive streak (Fig. 2d, i). By GD 7.5 (Theiler stage 11; presomite; corresponding to approximately 19-20 days post-fertilization in humans) the prechordal plate is readily identified as a circle of mono-ciliated cells that are subjacent to the rostral-most portion of the anterior neural plate (Fig. 2e, g). Caudal to the prechordal plate and extending to the position of the node, a line of mono-ciliated cells is intercalated into the ventral cell layer in the midline (Fig. 2e, g). *Shh* is expressed in a temporally-dependent manner in these axial populations [Echelard et al., 1993; Gunhaga et al., 2000]. Notably, the prechordal plate can be identified morphologically prior to the time in development when *in situ* hybridization indicates *Shh* gene expression. An early genetic marker of the prechordal plate is *goosoid* (*gsc*) [Filosa et al., 1997; Aoto et al., 2009] (Fig. 2f).

Figure 2h shows a dorsal view of a GD 7.5 mouse embryo from which the amnion has been removed to allow visualization of the neural plate. At this stage in development, the neural plate comprises nearly the entire anterior half of the embryo, with the prospective forebrain (prosencephalon) being at the rostral rim. Examining a midsagittal cut through an embryo of this stage allows appreciation of the relationships of the ventro-axial cell populations to the developing brain (Fig. 2i). Additionally informative is removal of ventral cell populations (endoderm, including the prechordal plate), which allows visualization of the mesenchyme (i.e., a non-epithelial, fibroblastic cell population) that is ventral to the developing brain. These mesenchymal cells are considered to be mesodermal (i.e. “of the middle germ layer”) and are derived, in part, from the primitive streak via gastrulation [Tam et al., 1993]. Some authors indicate that additionally, the prechordal plate is a source of cranial mesenchyme. For example, Müller and O’Rahilly [2003] state that the human prechordal plate transitions from a solid median structure to laterally migrating cells that form bilateral premandibular condensations.

Between the end of the 7th to the middle of the 8th day after fertilization in the mouse (Theiler stage 12; corresponding to approximately 20-22 days post-fertilization in humans) the first 7 pairs of somites form and the optic sulci that develop within the diencephalic portion of the forebrain become clearly distinguishable (Fig. 3). As previously shown in comparably-staged chick embryos (Fig. 1), the prechordal plate in early somite-stage mice presents as a compact mass of cells that is subjacent to the median aspect of the forebrain. This is readily seen in Figure 3b, which is an image of a mouse embryo from which a portion of the cranial neuroepithelium has been removed. Also illustrated is the association of the prechordal plate with a more laterally positioned mesenchymal cell population. At the late end of this stage of development, the prechordal plate is subjacent to the median diencephalic floor (Fig. 3c), with the developing eyes (optic sulci) positioned more laterally. At this time, the telencephalic portion of the forebrain (that part from which the cerebral hemispheres arise) is a very small component of the developing brain, making up only its rostral-most rim (Fig. 3c).

By the time that mouse embryos have approximately 8 somite pairs (Theiler stage 13; gestational day 8.5), *in situ* hybridization studies show that *Shh* expression is initiated in the neuroepithelium [Echelard et al., 1993]. It is first expressed in the ventral midline at the level of the midbrain, extending into the diencephalic portion of the forebrain and caudally into the hindbrain, such that by the time 15 somite pairs are present (approximately 12 hours later), expression is continuous in the ventral midline from the rostral limit of the diencephalon to the presumptive spinal cord.

Figure 4 includes images of both chick (a-c) and mouse embryos (d-i) at developmental stages that correspond to those occurring in humans during approximately the 24th to 26th days post-fertilization. During this time, the neural folds fuse, with the rostral closure site occurring at the junction of the telencephalic and diencephalic portions of the prosencephalon. Relative to the diencephalon, the telencephalon remains relatively small. In chick embryos, near the time of rostral neural tube closure (H&H 10-11), the prechordal plate is positioned at the apex of the foregut pocket, remaining continuous with the foregut endoderm and in close proximity to the floor of the diencephalon (Fig. 4a-c). Also in close proximity to the prechordal plate is the thickened surface ectoderm of Rathke's pouch, the progenitor of the anterior pituitary gland (Fig. 4c). By this time, the notochord has separated from the underlying endoderm, appearing as a median cellular cord that extends rostrally to the prechordal mesoderm. This morphology is very similar to that in comparably-staged mouse embryos (Theiler stage 14; corresponding to human embryos at approximately 24 days post-fertilization and occurring at 9 days of development in mice; Fig. 4d-f). By the time that mouse embryos reach Theiler stage 15 (9.5 days post-fertilization; corresponding to humans at approximately 26 prenatal days), 21-29 somite pairs are present, the oropharyngeal membrane has broken down and no longer separates the oral and foregut cavities, and the prechordal plate cannot be identified (Fig. 4g-i). In the midline, mesenchymal cells fill the space between the mesencephalic flexure and the roof of the foregut, and there is close apposition between the ectoderm of Rathke's pouch and the floor of the diencephalon.

Genesis of Teratogen-Induced HPE

A major aim of this overview is to illustrate the morphogenesis of a wide range of teratogen-induced brain and facial abnormalities that comprise the HPE spectrum. As described in a recent review by Cohen and Shiota [2002], HPE has been experimentally produced in a wide variety of non-mammalian and mammalian models by a number of different teratogenic agents. Dysmorphogenesis resulting from exposure of mouse embryos to a selected group of these teratogens during the critical period for HPE induction (GD 7-8.5) is described.

Among the earliest reports of experimentally-induced HPE were those in which ethanol was administered to chicks [Féré, 1899], frogs [LePlat, 1913] and fish [Stockard, 1910]. More recently, the genesis of a HPE spectrum resulting from exposure of mice to this teratogen has also been described [Sulik and Johnston, 1982; Sulik and Johnston, 1983; Webster et al., 1983; Sulik, 1984; Schambra et al., 1990; Godin et al., 2010]. In the mouse, treatment of dams with high doses of ethanol administered as 2 doses given 4 hours apart early on the 7th gestational day (when embryos are at the stages shown in Fig. 2a-d) results in the HPE phenotypes shown in Figures 5 and 6. In normal embryos having 29 somite pairs (late on GD 9), the olfactory placodes/developing nasal pits are evident and widely spaced on the frontonasal prominence (the tissue surrounding the telencephalon) (Fig. 5a). In ethanol-exposed embryos at this same developmental stage, the olfactory placodes are too closely spaced, accompanied by narrowing of the frontonasal prominence (Fig. 5 d, g). On GD 11, a time when the nasal pits should be surrounded by well developed lateral and medial nasal prominences (the progenitors of the nasal alae, and the nasal tip along with the intermaxillary segment, respectively), affected embryos present with medial nasal prominences that are abnormally small and closely approximated to the point of appearing as a single band of median tissue between nostrils that are too closely spaced (Fig. 5b, e, h). Deficiency in the median aspect of the forebrain accompanies that of the upper midface and is evidenced by abnormally close proximity (and in severe cases, median confluence) of the ganglionic eminences (Fig. 5f, j). Figure 6 illustrates a spectrum of the ethanol-induced facial phenotypes as they appear on GD 14 in mice. Mildly affected animals have facial features consistent with those in fetal alcohol syndrome, i.e. a small nose, deficient philtrum,

and a long upper lip, all of which are indicative of median tissue deficiencies (Fig. 6a, b). More severely affected mice may present with a single nostril, as in human cebocephaly (Fig. 6c, d), or with a median cleft lip associated with an absent intermaxillary segment (Fig. 6e, f). Notably, in teratogen-exposed mice, even in inbred animals, widely ranging degrees of effect commonly occur within single litters. This is attributable, at least in part, to the fact that there is significant intralitter variation in developmental stages [see Sulik and Johnston [1982] for details regarding the interrelationship of the developing face and brain in ethanol-induced HPE in the mouse].

While cyclopia/synophthalmia have not been noted to result from ethanol treatment on GD 7 in mice, mild to severe microphthalmia or anophthalmia occurs. While the eyes and forebrain remain sensitive to ethanol's teratogenicity when exposure occurs at later stages (extending through GD 9) [Kotch and Sulik, 1992; Parnell et al., 2009], HPE is an endpoint that appears to be specific to ethanol exposures that occur when gastrulation is just being initiated (GD 7 in the mouse). This temporal specificity for the induction of HPE with ethanol in mice is consistent with that for other species. Studies employing zebrafish as conducted by Blader and Strahle [1998] are particularly informative with respect to identification of a narrow window of vulnerability. These investigators showed that exposure of zebrafish embryos to ethanol over a time period as brief as 3 hours, encompassing late blastula and early gastrula stages, caused severe HPE. In the fish embryos, synophthalmia was a common endpoint.

Blader and Strahl's work has also shed light on the mechanisms involved in HPE induction. Using *goosoid* as a molecular marker for the prechordal plate (defined as that tissue that is derived from axial hypoblast cells that involute/ingress at the onset of gastrulation and migrate anteriorly ahead of the chordal mesoderm), they illustrated an abnormal caudal placement of this tissue and suggested that ethanol arrests its migration. Thus, absence of inducing tissue for the forebrain was considered to be the primary cause for the ethanol-induced HPE. Others have shown that in zebrafish, *shh* mRNA injection can prevent ethanol-induced cyclopia, suggesting that abrogation of Hedgehog (Hh) signaling is one of the major effects of ethanol exposure [Loucks and Ahlgren, 2009]. The specific cellular mechanism(s) by which ethanol perturbs this signaling remains to be definitively established as does the initial cell population and developmental event involved.

Another teratogenic agent that, when acutely administered on GD 7 in mice causes HPE is retinoic acid [Sulik et al., 1995]. As with ethanol, the result of exposure to this agent is a wide range of severity within the HPE spectrum. As shown in Figure 7, in addition to median upper face deficiencies, some of which are severe enough to yield proboscis formation (union of the lateral nasal prominences with absence of intermediate tissues), the developing lower jaw may be hypoplastic. Lower jaw deficiencies can also result from ethanol exposure on GD 7 [Godin et al., 2010], but are not illustrated herein. Typically, the maxillary component of the developing upper jaw appears to be much less affected than the mandibular region. That the mesenchyme of the maxillary prominences is almost entirely derived from neural crest cells, while that of the mandibular prominences has a large mesodermal contribution may account for this differential effect. The neural crest cells that contribute to the maxillary and mandibular prominences begin to migrate into those regions from the mid and upper hindbrain levels of the neural folds on GD 8 in the mouse, while the mandibular mesoderm is laid down at very early gastrulation stages; i.e. at or very near the GD 7 time of insult. Figure 7e illustrates an extreme example of loss of prechordal tissues. In this retinoic acid-exposed mouse fetus, the entire forebrain and associated facial tissues, along with the mandibular prominences are absent. However, the mid and hindbrain, along with the maxillary prominences are preserved.

Acute exposure to other teratogens on GD 8-8.5, when mice are at very early somite stages, also can yield defects in the HPE spectrum [Wei and Sulik, 1993; Lipinski et al., 2008]. Figure 8 illustrates defects caused by acute maternal treatment on GD 8 with Ochratoxin A (OA), a food-born mycotoxin. Some of the affected animals present with severe microphthalmia, while in others the eyes are less severely diminished in size, but very closely approximated, to the point of union (synophthalmia). In each of the specimens shown, virtually all of the medial and lateral nasal prominence tissues are absent. This is evidenced by median union of the maxillary prominences, producing a snout (not a proboscis) that is surrounded by rows of hair follicles. In some specimens with this facial morphology there was also failure of anterior neural tube closure, resulting in anencephaly (not shown). Study of the OA-mediated pathogenesis illustrated that within 6 hours of maternal drug treatment, a remarkable amount of cell death occurred in the presumptive telencephalon and forebrain floor [Wei and Sulik, 1993]. Excessive cell death in selected cell populations/regions was also notable at 24 hours after maternal treatment. The frontonasal prominence was particularly affected.

OA has also been employed in gene/environment interaction studies. Ohta et al [2006] have shown that in mice carrying a mutation in *Gli3* (a downstream gene in the Hh signaling cascade) which results in HPE, polysyndactyly, and neural tube defects, OA treatment on GD 7.5 increases the incidence of abnormalities. Additionally this group showed that in these mice, maternal folic acid administration at times surrounding the OA treatment reduces the incidence of teratogen-induced defects.

Also studying gene/environment interactions, Lanoue et al [1997] showed that in mutant mice in which cholesterol levels are low due to mutation of the *apolipoprotein b* gene, further reduction resulting from treatment with a cholesterol biosynthesis inhibitor induces HPE. In some individuals the forebrain and facial defects are accompanied by hindbrain and limb reduction abnormalities (Fig. 9) and in others the cranial neural tube fails to close (Fig. 10). In this model, the drug-induced hypocholesterolemia was accomplished by treating dams on their 4th through 7th day of pregnancy with the biosynthesis inhibitor. That the hindbrain and limbs (whose critical periods are after GD 7) were affected indicates that cholesterol remained reduced to teratogenic levels for some time beyond GD 7. Recognizing the requirement of cholesterol for normal Hedgehog signaling [Incardona and Roelink, 2000] Lanoue et al [1997] pointed out the similarities between the defects observed in their model and in *Shh* knockouts [Chiang et al., 1996]. More recently, Li et al [2007], have shown that in zebrafish, supplementing ethanol-exposed embryos with cholesterol rescues a loss of Shh signal transduction, and prevents embryos from developing HPE.

More directly examining teratogen-mediated insult to the Hh signaling pathway in mice, Lipinski et al [2008 and submitted] have employed cyclopamine and a potent synthetic analogue. These compounds inhibit the morphogenetic activity of the Hh pathway by binding to and preventing activation of the transmembrane protein, Smoothened (Smo) [Chen et al., 2002]. In the absence of Hh ligand, its receptor, Patched (Ptc1) inhibits Smo activity, presumptively through a small molecule mediator [Taipale et al., 2002; Bijlsma et al., 2006]. Upon Hh binding to Ptc1, inhibition of Smo is relieved, triggering a complex downstream signaling cascade that culminates in target gene activation via the Gli family of transcription factors [Ingham and McMahon, 2001]. As in the classic studies in which Binns and Keeler exposed sheep to cyclopamine [reviewed in Keeler, 1975] this agent and the analogue also cause the HPE spectrum in mice. In the Lipinski et al studies, maternal drug treatment was initiated on GD 8.25 - 8.5; i.e. close to the end of the known critical period for HPE induction. In addition to the range of defects described herein for other teratogens (e.g. cleft lip; Fig. 11a), unilateral and bilateral clefts of the lip as well as clefts of the secondary palate were induced (Fig. 11b, c). In some of the mice with cleft lip, the

intermaxillary segment is notably reduced in size and in others it appears normal. These phenotypes are also seen in clinical populations. In individuals in which intermaxillary segment reduction is recognized, median forebrain deficiencies are expected. However, in those that have what appears to be typical unilateral or bilateral cleft lip and palate, this is not the case. Noteworthy in this regard, is that histological sections of the cyclopamine-exposed mouse fetus featuring cleft lip and palate and shown in Figure 11 c revealed agenesis of the anterior pituitary (Fig. 12 f).

New Horizons in HPE Research and Prevention

Recent technological advances are greatly facilitating analysis of HPE-associated dysmorphology. Exemplary is high resolution magnetic resonance imaging (magnetic resonance microscopy; MRM) [Petiet et al., 2008]. With isotropic resolution in the range of 20-39 microns (near histological levels), a relatively rapid method for morphological assessments from individual scans is provided. Additionally allowed is creation of accurate 3-D reconstructions, facilitating shape and volumetric analyses as well as correlation of brain and facial dysmorphology in individual specimens [Parnell et al., 2009]. Using this methodology to study ethanol-induced defects in fetal mice, Godin et al [2010] have not only illustrated a broad spectrum of median forebrain insult, some examples of which are shown in Figure 13, but have also discovered that acute GD 7 ethanol exposure induces formation of cerebral leptomeningeal heterotopias (Fig. 14). This type of cortical abnormality is correlated to seizure activity in clinical populations [Verrotti et al., 2009]. It is remarkable that acute insult at a time when the neural plate is just being induced can yield this endpoint.

Another magnetic resonance-based methodology that offers considerable promise for advancing our understanding of HPE-associated defects is diffusion tensor imaging (DTI). This technique allows visualization of the pattern and integrity of CNS fiber tracts and is based on identifying the rate and direction of water diffusion through the tissue. Even in the absence of gross structural brain malformations, microstructural changes within the fiber tracts can be determined. Currently, DTI is being applied to the study of the brains of mice acutely exposed to ethanol on GD 7 [O'Leary-Moore, personal communication]. While, undoubtedly, a wealth of information will be provided from these DTI studies, technological advances that will allow reduction in the time required for small animal imaging are expected and will provide the potential to extend the analyses from fixed to live specimens, thus allowing correlation of morphological and behavioral phenotypes.

In addition to now being able to better define the features of the full HPE spectrum, the availability of a variety of teratogen-induced and genetically-based models along with advances in developmental biology research methodologies are making it possible to better understand the pertinent molecular and cellular mechanisms. With respect to teratogen-induced HPE, it will be of particular interest to further explore various mechanisms by which Hh signaling is directly or indirectly disrupted. While events occurring upstream (e.g. failure of Shh-expressing or responding cells to form) and downstream (e.g. failure of signal transduction) of Hh signal production, as well as those directly affecting the latter, may yield comparable endpoints, at least subtle differences are expected. Further studies to examine temporal specificities, the effects of drug exposure combined with gene manipulation, and insult resulting from combinations of HPE-inducing drugs are expected to provide important new insights into this etiologically complex spectrum.

For teratogen-induced HPE, understanding that the critical period for induction is in the 3rd to early in the 4th week of human gestation is, indeed, critical. Avoidance of the teratogenic

agents, as well as initiation of preventive measures, such as nutritional supplementation must occur prior to the time that most pregnancies are recognized.

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Biographies

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Figure 1.

A scanning electron micrograph (a, dorsal view) and a histological section (b) illustrate the normal cranial morphology of Hamburger and Hamilton (H&H) Stage 7 and 8 chick embryos, respectively. The dashed line in (a) illustrates the location of the sagittal section in (b). Subjacent to the developing forebrain (FB) and midbrain (MB) are columnar cells comprising the prechordal plate [PP, which is continuous with the foregut endoderm (FGE)] and mesenchymal (fibroblastic/non-epithelial) cells comprising prechordal mesoderm (PM), respectively. Caudal to the latter is the notochord (N), which underlies the hindbrain (HB). FG=foregut.

Figure 2.

Scanning electron micrographs illustrate the morphology of gestational day (GD) 7-7.5 (Theiler Stage 10, a-d; and 11, e-j) mouse embryos. As shown in (a) and at higher magnification in (b), at the tip of the ventral side of the cup-shaped embryo, cells having a relatively small surface area are intercalated into the endodermal layer. Small groups of these cells extend along the anterior midline of the embryo (arrows in b). Slightly later in development, these cells, which at higher magnification than shown, can be identified by the presence of a prominent mono-cilium, populate the anterior midline (arrows in c). A sagittal cut through an embryo of this stage (d) illustrates its cup-shaped form, with the ectodermal layer (including the neuroepithelium) lining the interior, and the amnion (open arrow) separating it from the extraembryonic components. The antero-ventral midline is indicated by arrows. The primitive streak and node (•) are located in the caudal midline. By GD 7.5, the position of the prechordal plate is notable on the ventral side of the embryo as a circular area (dashed arrow in e and white circle in f) that is located rostral to the notochoral plate (more caudally-positioned arrows). At this time, *Shh* is expressed in the cells of the antero-ventral midline, as indicated by the dark grey area in the schematic in (f), while *goosoid* is expressed in the prechordal plate (white circular region in f). Shown in (g) is an embryo that was cut at the level of the prechordal plate (dashed line in e). Illustrated is the columnar neuroepithelium of the forebrain (FB) and its proximity, in the midline, to the prechordal

plate (dashed arrows). A dorsal view of an embryo from which the amnion has been removed (h), shows the neural plate occupying the majority of the anterior half [region inside the dashed circle, including the forebrain (FB)]. As shown in (i), a midsagittal cut illustrates the relationships of the notochordal plate (solid arrows), the prechordal plate (dashed arrow), amnion (open arrow) and forebrain region (FB). At this developmental stage, mesodermal cells continue to be laid down as cells ingress through the primitive streak and node (}). Removal of the ventral cell layer on the left side of an embryo, including removal of the prechordal plate from the region shown by a dashed arrow, illustrates the subjacent cardiac and cranial mesenchyme (M) (j).

Figure 3.

Scanning electron micrographs illustrate the morphology of mouse embryos at Theiler stage 12, when the embryos are at approximately 8 days of gestation, and when 1-7 pairs of somite pairs are evident. The embryos in (a & b) are younger than that in (c). In the former the optic sulci (arrows in c) are not yet visible in the developing forebrain (FB) region of the neural plate. As shown in (b), removal of the epithelium of the left side of the developing brain, including that in the rostral midline, illustrates the subjacent prechordal plate (dashed arrow) and mesenchyme (M). In (c) the portion of the cranial neural plate that is the developing diencephalon is located between the two dashed black lines. The prechordal

plate is subjacent to the median aspect of this brain segment, as indicated by the dashed white shape. T = telencephalon.

Figure 4.

Images of H&H 10-11 chick (a-c) and Theiler stage 14-15 (GD 9-9.5) mouse embryos (d-i) illustrate relationships between the developing brain and subjacent tissues at the time of anterior neural tube closure (a-f) and shortly thereafter (g-i). In the chick, a sagittal cut through the anterior neuropore (open arrow) illustrates the notochord (arrowheads in b). At higher magnification, as shown in (c), the prechordal mesoderm (PM) can be identified rostral to the notochord; underlying the median diencephalon (D) is the prechordal plate (PP); the telencephalon (T) is rostral to the PP; and the ectoderm of Rathke's pouch (the progenitor of the anterior pituitary; arrow) is continuous with that of the buccopharyngeal membrane (dashed circle). The inner layer of the buccopharyngeal membrane is continuous

with the endoderm of the foregut (FG). In the mouse, at the time of anterior neuropore closure, while the developing forebrain is more ventrally positioned with respect to the remainder of the neural tube than in the chick, the overall morphology in these species is comparable (d-f). By the time that 3 pharyngeal arches (I, II, III) are clearly identifiable in the mouse, a midsagittal view illustrates that the PP can no longer be defined (g-i). Arrowheads in i = notochord; E= eye.

Figure 5.

Ethanol-induced dysmorphology in GD 9.5 and 11 mouse embryos following acute ethanol exposure on GD 7. Frontal views of the face of control (a, b) and ethanol-exposed embryos (d, e; g, h), along with views of the forebrain interior of the embryos in b, e, and h (c, f, and i) illustrate diminished median tissue in the affected embryos, with median apposition of the olfactory placodes/nasal pits (dashed circle) and ganglionic eminences (arrows) (d-i). Modified from [Sulik and Johnston, 1983; Sulik, 1984]

Figure 6.

Ethanol-induced dysmorphology in GD 14 mice following acute ethanol exposure on GD 7, and corresponding human phenotypes. Illustrated are children with Fetal Alcohol Syndrome (b), cebocephaly (d), and median cleft lip (f), whose features are represented in the spectrum of defects induced by acute maternal ethanol exposure in mice (a, c, d). Modified from [Sulik et al., 1988].

Figure 7.

Retinoic acid-induced dysmorphology in mouse embryos. As compared to control embryos (a, d), those whose mothers are treated with retinoic acid on GD 7 present with defects consistent with those shown in Figs. 5 & 6. In addition, retinoic acid exposure causes mandibular hypoplasia (M; b, c, e), and forebrain/ midface deficiencies that are severe enough to result in proboscis formation (representing median union of the lateral nasal prominences; arrow in c), or complete absence of facial features (with the exception of remaining maxillary tissue, open arrow) and aprosencephaly (e). Bars in a-c = 10 μ m. Modified from [Sulik et al., 1995].

Figure 8.

Ochratoxin A (OA)-induced dysmorphology in mouse fetuses. Illustrated are the holospheric forebrain (*) in a cecocephalic fetus (a, b), extreme hypotelorism (c), and synophthalmia (d), all of which followed acute maternal OA treatment on the 8th day of pregnancy. Note that the snout of each of these fetuses has several rows of hair follicles (arrows), which are indicative of maxillary prominence origin. Bars in a-d = 500 μ m. Modified from [Wei and Sulik, 1993].

Figure 9.

Cholesterol-deficiency-induced dysmorphology in mouse embryos. Comparison of a sagittal cut through the head of a control (a-c; b & c are reciprocal halves), and an affected embryo (d-f; f & g are reciprocal halves) illustrates deficiency in the forebrain, i.e. the portion of the brain that is rostral to the mesencephalic flexure (black dashed arrow), a narrow aqueductal isthmus (white dashed arrow), and a thickened hindbrain floor (*) in the latter. Bars in a, b, d, e = 5mm; in c, f = 100 μ m. Modified from [Lanoue et al., 1997].

Figure 10.

Anencephaly/HPE in the mouse and human. As shown in a mouse fetus with cholesterol deficiency-induced dysmorphia (a), and in a child with a similar presentation (b), facial features typical of HPE may be accompanied by anencephaly/ rostral neural tube closure failure (arrow). The arrowhead in (a) indicates a single nostril.

Figure 11.

Cyclopamine-induced dysmorphology in mouse fetuses and corresponding human phenotypes. A wide spectrum of dysmorphology including cebocephaly (a) and cleft lip (b, c) result from drug exposure initiated on GD 8.5. Notably, while the fetuses in b & c and the corresponding children present with bilateral cleft lip (e & f), the size of the intermaxillary segment (prolabium; arrows) differs. In the cebocephalic child shown in (d), although the lip is closed, there is no intermaxillary segment. Modified from Lipinski et al, submitted.

Figure 12.

Cyclopamine-induced CNS dysmorphology in fetal mice. As compared to frontal (coronal) histological sections made at the level of the eyes and pituitary in a control GD 16.5 fetus (a, b), abnormalities including union of the frontal lobes of the brain (open arrow in c) and complete pituitary aplasia are notable in a sections from the cebocephalic fetus shown Fig. 11 a (c, d), while a cleft palate (arrow in e), anterior pituitary agenesis and persistent posterior pituitary can be seen in sections from the fetus shown in Fig. 11c (e, f). Modified from Lipinski et al, submitted.

Figure 13.

Reconstructed high resolution magnetic resonance images illustrate normal brain morphology (a,b) and HPE (c-f) in fetal mice. The specimen shown in a dorsal and frontal view in (c, d), respectively, is asymmetrically affected. As in this specimen, that shown in (e, f) presents with rostral union of the cerebral hemispheres, while the more caudal aspects of the brain appear relatively normal. Pink = olfactory bulbs, Red = cerebral hemispheres, Light green = diencephalon, Magenta = midbrain, Dark green/teal = cerebellum, Medium green = myelencephalon. Modified from [Godin et al., 2010].

Figure 14.

Cerebral cortical heterotopias in fetal mice with holoprosencephaly. Histological sections of the cebocephalic fetus whose face is reconstructed from magnetic resonance images and shown in (a) illustrate cerebral cortical leptomeningeal heterotopias (arrows in b). The boxed area in (b) is shown at higher magnification in (c).