Cadherins in the Developing Central Nervous System: An Adhesive Code for Segmental and Functional Subdivisions

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In this review, we describe general features of the expression of cadherins in the developing central nervous system (CNS) of vertebrates. In the early neuroepithelium, the expression of several cadherins is restricted to specific regions corresponding to segmental domains. Segmental boundaries often coincide with changes in cadherin expression, subdividing the primordial CNS into different adhesive domains. In the different neuronic domains, early neurons are generated which differentially express cadherins. In the mantle layer, these early neurons seem to sort out according to which cadherin they express, and they aggregate into various gray matter regions (brain nuclei and cortical lamina and regions). The gray matter structures expressing a given cadherin become connected to one another to form parts of particular functional systems or neuronal circuits. Together, these findings show that cadherins provide a molecular system reflecting both early embryonic and mature nervous system architecture. The possible roles of cadherins in the formation and maintenance of segmental and functional nervous system structures are discussed.

INTRODUCTION

In its complexity, the vertebrate nervous system is unmatched by any other organ. To elucidate the molecular and cellular mechanisms controlling its development is a major challenge. Already at the gross morphological level, the nervous system displays an enormously complicated architecture. It is composed of a large variety of distinct groups of neural cells (“gray matter”) which are connected to each other by nerve cell processes (“white matter”) in a precise and specific manner. While the histology and functional connectivity of the different regions in the nervous system have been studied by neuroanatomists for more than a century, we are just beginning to understand the molecular basis of their development. For example, in recent years, the molecular mechanisms underlying axon guidance have attracted much attention, and important findings and concepts have been put forward. Other topics have been less intensively studied, such as the formation of gray matter structures.

In the present review, we discuss developmental mechanisms involved in the establishment and maintenance of functional nervous system anatomy, with a particular focus on the segmental subdivisions of the early embryonic brain, and the formation of brain nuclei, cortical layers, and neuronal circuits. We will present recent evidence suggesting that a family of cell–cell adhesion molecules, the cadherins, may provide a key to understand the molecular basis for the formation of these structures.

Cadherins are cell surface glycoproteins which confer differential adhesiveness to embryonic cells and regulate morphogenesis in many organs in a calcium-dependent fashion (reviewed in Takeichi, 1988, 1995; Ranscht, 1994). More than 10 members of the cadherin family of molecules are expressed in the vertebrate brain (reviewed in Redies, 1995). Based on their molecular structure, cadherins can be grouped into classic cadherins (type I and II), desmosomal cadherins, protocadherins, and other types of cadherins (Suzuki et al., 1991; Sano et al., 1993; Ranscht, 1994; Tanihara et al., 1994). A characteristic feature of cadherins is that they prefer to bind to their own type (“homophilic binding”) (reviewed...
in Ranscht, 1994; Takeichi, 1995). Heterophilic binding between different types of cadherins has been described but is generally weaker (Inuzuka et al., 1991a; Matsunami et al., 1993; Murphy-Erdosh et al., 1995; Nakagawa and Takeichi, 1995). As a consequence of the preferential, homophilic binding, cells expressing the same cadherin aggregate or associate with each other and sort out from cells expressing other types of cadherin. Moreover, cells expressing the same cadherin in different amounts also segregate (Steinberg and Takeichi, 1994). It is this concept of qualitative and quantitative differences in cellular adhesiveness (Moscona and gene products (Simeone et al., 1987; Krumlauf et al., 1993; Murphy-Erdosh et al., 1993; Bulfone et al., 1993; Figdor and Stern, 1993; Macdonald et al., 1994; Stoykova and Gruss, 1994).

We recently showed that several cadherins (E-cadherin, R-cadherin, cadherin-6, cadherin-8, and cadherin-11) are expressed in a restricted fashion in the early neurogenic chicken and mouse brain (Gänzler and Redies, 1995; Matsunami and Takeichi, 1995; Kimura et al., 1996; T. Inoue and M. Takeichi, unpublished; K. Korematsu and C. Redies, unpublished). A similarly restricted expression was reported for R-cadherin in the embryonic frog brain (Espeseth et al., 1995). Each of these cadherins shows a unique expression pattern consisting of several stripes of expression which can be related to the segmental organization of the early embryonic brain. To illustrate this point, Fig. 1A shows the expression of R-cadherin protein in a whole mount preparation of Embryonic Day 12.5 (E12.5) mouse brain (Matsunami and Takeichi, 1995). R-cadherin is expressed in several patches distributed throughout the brain from the telencephalon to the hindbrain. Strikingly, the borders of cadherin expression are often sharp and coincide with neuromere boundaries. For example, the expression of R-cadherin in the alar plate of the ventral thalamic neuromere (vt) falls off at the zona limitans intrathalamica which is the boundary region to the dorsal thalamic neuromere (dt). Rostrally, another sharp border of expression is seen at the boundary to the ganglionic eminence. This border (not shown in Fig. 1A) extends into the telencephalon and likely corresponds to a segmental boundary (Puelles and Rubenstein, 1993; Macdonald et al., 1994). The neuromeric expression pattern of R-cadherin in the embryonic chicken forebrain (Gänzler and Redies, 1995) is similar (but not identical) to that in the mouse.

Other examples for the restriction of cadherin expression to embryonic subdivisions are found in the hindbrain and in the telencephalon of the early mouse embryo. In the hindbrain, cadherin-6 is expressed throughout the neural plate with a sharp anterior limit at the future rhombomere 4/5 boundary at E8. At E8.5, the expression has become confined to rhombomere 6 (T. Inoue and M. Takeichi, unpublished). In the telencephalon, R-cadherin and cadherin-8 are expressed in the cortical neuroepithelium with a sharp border of expression to the adjacent ganglionic eminence (Matsunami and Takeichi, 1995; K. Korematsu and C. Redies, unpublished). A complementary expression pattern was found for cadherin-6 which is expressed by the gangli-

**BRAIN SEGMENTS AND THEIR SUBDIVISIONS**

The nervous system derives from neural tube which is formed during neurulation when the surface ectoderm folds in at the dorsal midline. The two lateral ridges of the fold fuse to form a hollow tube which separates from the surface ectoderm during neural tube closure. While the surface ectoderm expresses E-cadherin (or L-CAM), the prospective neural tissue switches its expression to N-cadherin (Thiery et al., 1984; Nose and Takeichi, 1986). Unlike most other cadherins (see below), N-cadherin continues to be ubiquitously expressed in the undifferentiated neuroepithelium (Hatta et al., 1987). We recently showed that another cadherin, cadherin-6B, is expressed in the lateral ridges of the neural fold in the area of fusion (Nakagawa and Takeichi, 1995). This finding suggests that this cadherin, together with N-cadherin, may play a role in early neural tube formation.

The wall of the neural tube consists of a sheet of undifferentiated "neuro" epithelial cells. During development, this tube forms several distensions at its rostral end (the "brain vesicles"). Before acquiring its mature architecture, the nervous system undergoes a process of pattern formation resulting in the establishment of transverse and longitudinal segmental subdivisions within the neuroepithelium (the "neuromeres" and their subdomains) (reviewed in Puelles et al., 1987; Krumlauf et al., 1993; Puelles and Rubenstein, 1993; Guthrie, 1995).

Although brain segmentation has been known for more than a century, its developmental significance has recently been reappraised when it became clear that the molecules and general mechanisms involved in nervous system segmentation are similar to those controlling early pattern formation in the invertebrate embryo. In both systems, the segmental organization is reflected in a relatively simple map of embryonic fields bordering each other, with each embryonic subdivision characterized by the expression of a specific combination of gene regulatory proteins. For example, in the hindbrain, segment identity can be determined on the basis of the expression of Hox genes (Lumsden and Keynes, 1989) and, in the forebrain, several other gene families with expression patterns restricted to particular segments are found, such as the Emy, Eph, Otx, Pax, and Wnt gene products (Simeone et al., 1992; Boncinelli et al., 1993; Bulfone et al., 1993; Figdor and Stern, 1993; Macdonald et al., 1994; Stoykova and Gruss, 1994).
In some areas where the boundaries themselves contain cells, these cells express particular cadherins (Fig. 2). For example, the zona limitans intrathalamica expresses E-cadherin and cadherin-6, -8, and -11 while the immediate surroundings can be negative for these cadherins (Matsunami and Takeichi, 1995; Kimura et al., 1996; T. Inoue and M. Takeichi, unpublished). Similarly, F-cadherin is expressed at the boundaries between several of the major subdivisions of the frog brain, e.g., at the boundaries between the alar and basal plates (Espeseth et al., 1995). The floor and roof plates which separate the two halves of the CNS along the midline also express cadherins in a pattern unique for each cadherin (Inuzuka et al., 1991b; Shimamura and Takeichi, 1992; Redies et al., 1993; Kimura et al., 1996).

The expression maps of five cadherins in the ventricular layer of the E12.5 mouse brain are displayed in Fig. 2. These maps demonstrate that the neuroepithelium of the early embryonic brain is subdivided into domains of differential adhesiveness which correspond to its segments and their subdivisions. Although each cadherin has a unique expression pattern, several embryonic subdivisions express multiple cadherins in a combinatorial fashion.

The following morphoregulatory roles for cadherins in the segmented brain are possible. First, by conferring differential adhesiveness to cells in adjacent embryonic subdivisions and/or to cells within boundary regions, the neurogenic pattern established by the expression of gene regulatory proteins could be stabilized (Espeseth et al., 1995; Gänzler and Redies, 1995; Matsunami and Takeichi, 1995). Generally, only a small minority of neuroepithelial cells can cross segmental boundaries (Fraser et al., 1990; Figdor and Stern, 1993; Fishell et al., 1993; Birgbauer and Fraser, 1994) at developmental stages when cadherin expression is a prominent feature. Moreover, cells derived from different embryonic forebrain subdivisions tend to sort out when re-aggregated in vitro (Whitesides and LaMantia, 1995). It has been hypothesized that this lack of mixing is due to the differential expression of adhesive factors in adjacent subdivisions (Lumsden, 1990). Cadherin expression may provide the molecular basis for this finding. For example, it was shown that dissociated primary cells from early embryonic mouse brain expressing R- or E-cadherin segregate in vitro according to which cadherin they express (Matsunami and Takeichi, 1995). This sorting is mediated by a calcium-dependent adhesion mechanism. Moreover, the aggregation of E-cadherin-expressing cells can be specifically blocked by antibodies against this molecule.

Second, the differential expression of cadherins in the neurogenic domains may provide a frame of reference for the subsequent generation of gray matter structures in the mantle layer (Redies, 1995). By generating cadherin-positive populations of early neurons in distinct but overlapping neurogenic subdomains (see below), initial coordinates for their subsequent sorting and aggregation in the mantle layer may be provided.

**FIG. 1.** Examples of R-cadherin expression by neurones at early stages (A) and by brain nuclei at intermediate stages of development (B). (A) Whole mount immunostaining of E12.5 mouse brain with an antibody against R-cadherin. A view onto a mouse brain cut in the mid-sagittal plane. Note that the expression in the ventral thalamus (vt) sharply declines at the border to the dorsal thalamus (dt) (arrowheads). Expression is also prominent in the pretectal area (pt) with a border to the mesencephalon (mes) (arrows). The basal plate shows little or no R-cadherin expression. Expression is also prominent in the prefrontal area (pt) with a border to the mesencephalon (mes) (arrows). The basal plate shows little or no R-cadherin expression. Compare the expression pattern to the schematic diagram shown in Fig. 2C. The asterisk indicates the position of the optic stalk. Staining by in situ hybridization shows a similar pattern of R-cadherin localization. (B) In situ hybridization of R-cadherin transcript on a frontal slice from E16 chicken diencephalon. The dark precipitate represents R-cadherin expression. Note the restricted expression in particular brain nuclei. Data are from the study by Matsunami et al. (1995) (A) and were kindly provided by N. Uchida (B), respectively. ALa, nucleus ansae lenticularis anterior; cb, cerebellum; dt, dorsal thalamus; mes, mesencephalon; PT, nucleus pretectalis; ROT, nucleus rotundus; T, nucleus triangularis; tel, telencephalon; TeO, tectum opticum; VMN, nucleus ventromedialis hypothalami; vt, ventral thalamus. Scale bars, 500 μm.
Third, as a consequence of the increased expression of particular cadherins by the boundary regions and in their immediate vicinity, ridges, flexures, or sulci may form at these locations on the ventricular surface of the brain during different times of development (Puelles and Rubenstein, 1993; Espeseth et al., 1995; Gänzler and Redies, 1995; Matsunami and Takeichi, 1995). Similar morphogenetic changes are regulated by cadherins in developing tissues of other organs (reviewed in Takeichi, 1988; Takeichi, 1995). The functional significance of these morphogenetic changes in the brain is unclear.

**CADHERIN EXPRESSION BY NEUROEPITHELIAL CELLS AND EARLY NEURONS**

What cells in the different neuromeres are expressing cadherins? Two main types of cell can be distinguished in the early neuromeric brain: neuroepithelial cells and early neurons. Neuroepithelial cells are proliferative, and their cell bodies are located in the ventricular layer close to the ependymal lining of the ventricles. Many neuroepithelial cells are bipolar and extend one process to the ependymal lining and another to the outer ("pial") surface ("radial glia"). The neuroepithelial cells give rise to the early neurons which, after their final mitosis, migrate toward the outer surface of the brain where they form the mantle layer. As development proceeds, more and more neurons accumulate in the mantle layer, which gradually increases in thickness. In the mantle layer, the neurons differentiate further to give rise to the various gray and white matter structures of the mature brain.

Cadherins are expressed by neuroepithelial cells and by early neurons. Which cell type expresses a given cadherin depends on the specific region, the cadherin, and the developmental stage. As an example, the cell types expressing R-cadherin in the E5 chicken hypothalamus are shown in Fig. 3. In some regions, a particular cadherin is predominantly expressed by undifferentiated neuroepithelial and/or radial glial cells (Figs. 3A and 3D), as demonstrated for E- and R-cadherin by immunohistochemistry (Shimamura and Takeichi, 1992; Gänzler and Redies, 1995; Matsunami and Takeichi, 1995). For cadherin-8 and -11, transcripts are found in cell bodies located in close proximity to the ependymal lining of the ventricular layer, indicating that these cadherins are also expressed by neuroepithelial cells and/or radial glia in these regions (Kimura et al., 1996; K. Korematsu and C. Redies, unpublished). In the mouse hypothalamus, cadherin-11 mRNA expression was shown to remain restricted to the ventricular layer at all developmental stages (Kimura et al., 1996). However, in the E3–E6 chicken brain, R-cadherin-positive cells are eventually found in all mantle layer regions which show expression by neuroepithelial cells (Gänzler and Redies, 1995).

As discussed above, the borders of cadherin expression by neuroepithelial cells often coincide with segmental boundaries. Many of the undifferentiated cells in the ventricular layer are radial glia. In the chicken and mouse brain (see Figs. 3A and 3D) (Shimamura and Takeichi, 1992; Gänzler and Redies, 1995; Matsunami and Takeichi, 1995), E- and R-cadherin-positive radial glial processes were shown to form dense palisades which span the entire thickness of the neural tube. Such palisades expressing different cadherins at each side of the segmental boundaries could restrict cell migration and mixing not only in the ventricular but also in the mantle layer. Moreover, localized sets of radial glial fibers expressing particular cadherins may serve as adhesive substrates for the outward migration of specific populations of newly generated neurons, as was suggested for E- and R-cadherin (Shimamura and Takeichi, 1992; Gänzler and Redies, 1995). Another function of cadherins may be the confinement of undifferentiated neuroepithelial cells to the ventricular layer (Barami et al., 1994; Kimura et al., 1996).

In other regions, a particular cadherin is not expressed by neuroepithelial cells but by a subpopulation of early differentiating neurons in the anlage of brain nuclei (see, e.g., Figs. 3A and 3B) (Gänzler and Redies, 1995; Kimura et al., 1996; K. Korematsu and C. Redies, unpublished). It has been shown for N- and R-cadherin in the chicken brain that this type of expression can be observed from the earliest stages of mantle layer formation. In some areas, newly generated neurons can be found in the outer zone of the ventricular layer during their migration to the mantle layer (Gänzler and Redies, 1995) (Figs. 3A and 3C).

**CADHERIN EXPRESSION IN THE DEVELOPING MANTLE LAYER**

Brain segmentation is a transient phenomenon observed early in development. As development proceeds, the segmented neuroepithelial wall of the neural tube transforms into the three-dimensional architecture of the mature CNS which can be subdivided according to a different principle: In order to simultaneously process different modalities of information, the CNS consists of functional systems which are each adapted to carry out a particular information processing task(s) (e.g., visual system, motor system, limbic system, etc.). Each system is composed of several gray matter areas that are derived from different neuromeres throughout the nervous system and are connected by fiber tracts, sometimes over wide distances, to form neuronal circuits. Mature brain architecture thus represents a complex type of organization globally spanning the entire nervous system, whereas segmentation represents a relatively simple, local principle of organization.

**Gray Matter Structures**

Most cadherins studied so far are differentially expressed by particular subpopulations of early neurons in the mantle
FIG. 2. Schematic expression maps for five cadherins in the E12.5 mouse brain (B±F). A schematic diagram of embryonic subdivisions and their boundaries is shown in (A). The colored areas indicate expression by the ventricular layer. Note that cadherin expression by areas of the mantle layer is not indicated in these diagrams. The thick colored lines indicate expression by boundary regions. The saturation of color roughly indicates expression levels. The position of the alar/basal plate boundary as described by Bulfone et al. (1993) is marked by the red line in (A). Data are from the studies by Matsunami et al. (1995) (B, C), T. Inoue and M. Takeichi (unpublished) (D), K. Korematsu and C. Redies (unpublished) (E), and Kimura et al. (1996) (F). A/B, alar/basal plate boundary; C, cerebral cortex; Cb, cerebellar anlage; DT, dorsal thalamus; ET, epithalamus; FP, floor plate; GE, ganglionic eminence; is, isthmic region; Mes, mesencephalon; OS, optic stalk; PT, pretectal areas; r1-r8, rhombomeres 1-8; RP, roof plate; VT, ventral thalamus; and zl, zona limitans intrathalamica.
FIG. 3. Example of different cadherin-expressing cell types in the brain of the chicken embryo. A cross section through the wall of the hypothalamic area of an E5 embryo immunostained with antibody against R-cadherin is shown in (A) together with schematic diagrams of the different cell types expressing R-cadherin in (B–D). Neuroepithelial and radial glial cells (D) have cell bodies located in the subventricular zone. Their processes reach the pial surface. Note the dense meshwork of radial processes and the sharp border of R-cadherin expression extending from the ependymal to the pial surface (arrowheads in A and D). Cell bodies of newly generated neurons are found in the mantle layer and, in a few areas, also in the outer zones of the ventricular layer (C), possibly during their radial migration. Their processes extend into the mantle layer but do not reach the pial surface. Some of their processes reach the ependymal lining, where they form an endfoot. Differentiating neurons aggregate in the anlagen of brain nuclei of the mantle layer (B) and extend cadherin-positive neurites which form fiber bundles (fb in A, B). Note that different R-cadherin-expressing cell types coexist at this timepoint of development. Data shown in (A) are from the study by Gänzler and Redies (1995). Scale bar in (A), 50 μm.
cadherin-expressing populations, the aggregates then assume the various morphologies of mature gray matter structures. It has been proposed that the forced driving cell sorting is free binding energy which tends to be minimized during morphogenesis (Steinberg, 1963). Combinatorial expression of several cadherin types and (weaker) heterotypic interactions between cells expressing different cadherin types may increase the complexity of the anatomical structures formed.

In general, at intermediate stages of development, prominent morphological borders between neighboring gray matter areas coincide with prominent changes in cadherin expression, whereas morphological gradients go hand in hand with gradients of cadherin expression (Arndt and Redies, 1996). In contrast, at later stages of gray matter development, cadherin expression can be sharply regionalized within a particular layer or nuclear subregion without obvious morphological boundaries to adjacent areas. For example, in the developing cerebellar cortex, there are parasagittal stripes of expression for several cadherins in chicken and mouse (Arndt and Redies, 1996; K. Korematsu and C. Redies, unpublished; S. C. Suzuki and M. Takeichi, unpublished). These stripes have no clear morphological correlations but it is possible that they coincide with cerebellar innervation patterns although this hypothesis needs to be experimentally verified. Likewise, in some nuclei which contain functional subdivisions, the expression of R-cadherin was shown to become restricted to particular subdivisions during the late differentiation of these nuclei (Arndt and Redies, 1996). It is therefore likely that, besides their morphoregulatory roles during intermediate stages of development, cadherins have other functions during late stages of gray matter differentiation and in the mature brain.

Nerves and Fiber Tracts

Not only gray matter structures but also developing white matter tracts and peripheral nerves (or parts thereof) differentially express particular cadherins throughout the developing nervous system (Shiga and Oppenheim, 1991; Redies et al., 1992, 1993; Shimamura et al., 1992; Shimamura and Takeichi, 1992; Fredette and Ranscht, 1994; Uchiyama et al., 1994; Arndt and Redies, 1996). Within a fiber tract or nerve, populations of neurites expressing a particular cadherin often form separate neurite fascicles. Such neurites often have a course within the CNS which differs from that of neighboring fascicles expressing another cadherin. Examples for the differential expression of cadherins by neurite fascicles are shown in Fig. 4. Immunoelectron microscopic analysis showed that E-cadherin is present where the surfaces of the neurites within each fascicle contact each other and that these fascicles segregate from E-cadherin-negative neurites (Uchiyama et al., 1994) (Fig. 4A).

There are several possible roles for cadherins in neurite outgrowth. First, cadherin expression by neurites is particularly strong at the time of axon elongation and tract formation and it is later reduced in many tracts when neurites have reached their targets. Since at least N- and R-cadherin were shown to provide excellent substrates for neurite elongation (Bixby et al., 1987; Matsuura et al., 1988; Neugebauer et al., 1988; Tomaselli et al., 1988; Bixby and Zhang, 1990; Drazba and Lemmon, 1990; Redies and Takeichi, 1993b), and the expression of these molecules is restricted to particular fiber tracts and segmental boundaries (see above), it is possible that cadherins serve as homophilic guidance molecules for the navigation of neurites along pre-existing neurites (Redies et al., 1992) or along the glial paths at segmental boundaries that express the same molecule. Interestingly, some of the earliest fiber tracts grow along segmental boundaries (Lumsden and Keynes, 1989; Figdor and Stern, 1993; Wilson et al., 1993). N-cadherin was shown to be expressed by growth cones (Letourneau et al., 1990; Shiuya et al., 1994). For T-cadherin, it was postulated that this molecule may represent a repulsive molecule for neurite outgrowth (Fredette and Ranscht, 1994). In this context, the differential expression of cadherins by the floor and roof plates (see Fig. 2) is interesting because these structures provide barriers for some neurite populations while allowing others to pass across the midline (Inuzuka et al., 1991b; Shiga and Oppenheim, 1991; Redies et al., 1993; Fredette and Ranscht, 1994). Second, cadherins may play a role in selective neurite fasciculation. E-cadherin is localized at the contact sites between particular populations of fasciculated sensory neurites in the mouse brain (Fig. 4A) (Uchiyama et al., 1994) and N-cadherin was shown to be expressed at axon-axon contact sites of regenerating peripheral nerves in the chicken (Shiuya et al., 1995). Inhibition of N-cadherin by functionally blocking antibodies causes defasciculation of N-cadherin-expressing neurites (Drazba and Lemmon, 1990; Redies et al., 1992). Third, cadherins may be involved in some aspect of target recognition since the targets of cadherin-expressing neurites often express the same type of cadherin (Redies et al., 1993; Arndt and Redies, 1996).

Functional Systems and Neuronal Circuits

By immunohistochemical analysis of N- and R-cadherin expression in the chicken embryo, we showed that the N- and R-cadherin-expressing fiber tracts connect some of the gray matter structures expressing the same cadherin (Redies et al., 1993; Arndt and Redies, 1996). Functional connectivity of the gray matter regions expressing particular cadherins was also indicated by in situ hybridization results for N-cadherin and cadherin-8 and -11 in the mouse embryo (Redies and Takeichi, 1993a; Kimura et al., 1996; K. Korematsu and C. Redies, unpublished). Although the in situ hybridization analysis did not directly visualize cadherin expression by the connecting fiber tracts between positive gray matter structures, their connectivity was previously reported in the neuroanatomical literature.

The expression of each cadherin studied so far is not restricted to only one particular functional system but to particular neuronal circuits within several systems, although...
FIG. 4. Cadherin expression by neurite fascicles. (A) Immunoelectron microscopy with antibodies against E-cadherin visualized by horseradish peroxidase-conjugated secondary antibody. This section shows a region of lamina II in the dorsal horn of a 28-day-old mouse spinal cord. E-cadherin expression is indicated by the dark precipitate. Note its presence between the axonal membranes of unmyelinated fibers of small diameter which are grouped in small fascicles (arrows). These fibers likely mediate pain and temperature sensation. (B, C) Double-label immunohistochemistry with antibodies against N-cadherin (B) and R-cadherin (C). This cross section shows vagus nerve fascicles at their entry into the hindbrain (hb) of the E8 chicken embryo. Arrows point at an N-cadherin-expressing fiber bundle. Its fibers join the descending (spinal) trigeminal tract (dV) to project caudally. They are likely to mediate general somatic sensation. Arrowheads point at fiber bundles expressing R-cadherin. Its fibers project or originate more medially and are likely to represent visceral (sensory and motor) fibers. Results shown in (B) and (C) are from the same section. Data are from the study by Uchiyama et al. (1994) (A) and Redies et al. (1993) (B, C). Scale bar, 50 μm (B, C).

One system may predominate for a given cadherin. For example, N-cadherin expression is most prominent in visual and limbic system structures in chicken and mouse (Redies et al., 1993; Redies and Takeichi, 1993a), whereas several circuits associated with motor control and the visual system express R-cadherin in the chicken (Arndt and Redies, 1996). Two of these R-cadherin-positive circuits are schematically shown in Fig. 5. Other parts of the visual and motor systems do not express N- or R-cadherin and both molecules are also found in specific functional elements and circuits of other systems. Taken together, these results show that members of the cadherin family belong to a class of molecules which can be used as markers for functional CNS circuitry (Redies et al., 1993; Redies, 1995).

The role of cadherins in establishing neuronal connections over large distances in the brain is unclear. Cadherins are likely to be involved in some specific aspects of circuit formation, e.g., in axon guidance, axon fasciculation, or target recognition, as discussed above. Other molecules expressed in a restricted fashion by particular neural circuits surely also contribute to these processes.

CADHERIN EXPRESSION BY NEURAL CREST DERIVATIVES

The development and differentiation of neural crest cells resemble that of neural cells in the CNS. Both types of cells derive from relatively homogeneous populations of undifferentiated cells and, upon differentiation, their progeny undergo a sorting process and aggregate into diverse morphological and functional structures. Like early neurons in the CNS, neural crest cells migrate before they become sessile.
Recent evidence suggests that cadherins are not only involved in nervous system compartmentation, but also in neural crest migration and sorting. During migration, subsets of neural crest cells take different paths and differentiate into particular tissues. These differentiating neural crest populations express different cadherins. For example, early sensory ganglia express N-cadherin (Inuzuka et al., 1991b; Redies et al., 1992), sympathetic and parasympathetic ganglia express N- or R-cadherin (Inuzuka et al., 1991b), and Schwann cells express cadherin-6 and -7 (Nakagawa and Takeichi, 1995; T. Inoue and M. Takeichi, unpublished). Interestingly, the expression of these cadherins can already be observed in neural crest cells during their migration when cells still assume a mesenchymal morphology (Nakagawa and Takeichi, 1995).

**CONCLUSION**

During the development of the vertebrate nervous system, several cadherins are differentially expressed in a restricted fashion by subpopulations of neural cells. Early in development, the distribution of these populations reflects the relatively simple segmental structure of the primordial neuroepithelium. Later in development, the expression reflects the complex functional anatomy of the vertebrate brain. We propose that cadherins have several morphoregulatory functions in the maintenance of segmentation and in the gradual emergence of functional structures such as brain nuclei, cortical layers, and neural circuits from the primordial neuroepithelium. These functions are likely to be based on qualitative and quantitative differences in adhesiveness between neural cells expressing cadherins and other adhesion molecules and include cell sorting and cell aggregation mechanisms. Such mechanisms have been known for decades (see article by M. Steinberg in this issue). Their central role in the development of nervous system anatomy is recently becoming clearer as the molecules regulating these processes are being identified.

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