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Local and transient expression of E-cadherin involved in mouse embryonic brain morphogenesis

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Summary

We found that E-cadherin (uvomorulin) is transiently expressed in restricted regions of the metencephalon, mesencephalon and diencephalon of mouse embryonic brain. This expression first occurred in parts of the mesencephalon and diencephalon at around E9.5, and subsequently extended to the primordia of cerebellum, the dorsal midline of mesencephalon and some other regions of the embryonic brain. These E-cadherin expressions ceased by E15 except at the dorsal midline. Immunohistological analyses showed that E-cadherin-positive cells are radially arranged in the neural tube and the E-cadherin-positive regions are sharply demarcated from E-cadherin-negative regions. Axons extending from some of the E-cadherin-positive regions also expressed this molecule. When embryonic brains were dissociated into single cells and cultured as monolayers, E-cadherin-positive cells formed clusters that were segregated from E-cadherin-negative cells. E9.5 brain fragments containing metencephalon and mesencephalon were isolated, explanted on Nuclepore filters and cultured in the absence or presence of antibodies to E-cadherin. This antibody treatment removed most of the E-cadherin molecules from the explants and consequently affected their growth pattern. To analyze cellular events induced by the antibody treatment, we stained these explants with an antiserum to *En* whose distribution was found to overlap in part with that of E-cadherin and found that the pattern of *En* staining was altered by the anti-E-cadherin antibody treatment. These results suggest that the local and transient expression of E-cadherin in embryonic brain is involved in regional pattern formation in this organ.

Key words: E-cadherin, *En*, brain morphogenesis, organ culture, mouse embryo.

Introduction

Morphogenesis of the brain is regulated by a complex series of cellular interactions. One of these processes, the specific adhesion of cells, is assumed to be important. Various classes of adhesion molecules have been identified in the nervous system and they are thought to play crucial roles in directed axonal migration, specific connections of neurons and many other neural cell behaviors (Hynes and Lander, 1992).

Cadherins are a family of cell-cell adhesion receptors with more than 15 members (Magee and Buxton, 1991; Ranscht, 1992). Of these, at least 10 members, including N, R, B and T-cadherins, are differentially expressed in the nervous system (Suzuki et al., 1991; Inuzuka et al., 1991; Napolitano et al., 1991; Ranscht and Dours-Zimmermann, 1991; Redies et al., 1992). Some of these molecules have been shown to be crucial for selective adhesiveness of cells (Nose et al., 1988; Miyatani et al., 1989) and their possible roles in the sorting of neural cells have been suggested (Takeichi et al., 1990; Redies et al., 1992; Shimamura et al., 1992).

The cadherin-mediated cell-cell adhesion seems also to be important in maintaining specific functions of cells. For example, embryonic skin rudiments cannot undergo normal morphogenesis in the presence of antibodies to cadherins (Gallin et al., 1986; Hirai et al., 1989b). In this case, blocking of the cadherins expressed in the epidermis leads to inhibition of mesodermal cell condensation, implying that the epidermal cells fail to send morphogenetic signals to the mesoderm when their cadherin-mediated adhesion has been destroyed. It is also known that cadherins are essential in establishing polarity in a cell. Introducing exogenous cadherins into L cells results in the relocalization of Na^+, K^+-ATPase into the basolateral cell surfaces; otherwise, this enzyme is distributed all over the surface of these fibroblastic cells (McNeill et al., 1990). Thus, cell-cell adhesions via cadherins appear to be crucial for eliciting a variety of cell functions. To investigate whether such cadherin actions are involved in neural morphogenesis should be intriguing.

E-cadherin is a member of the cadherin family which is generally important for epithelial cell-cell adhesion (Takeichi, 1988, 1991). In early embryos, the entire ectoderm expresses E-cadherin, but this molecule disappears from the

region of the ectoderm that is differentiating into the neural tube (Nose and Takeichi, 1986). Our recent study, however, revealed that a subset of sensory neurons express E-cadherin, indicating that this cadherin is not excluded from the nervous system (Shimamura et al., 1992). In the present study, we found that E-cadherin is transiently expressed in parts of the mouse embryonic brain. We analyzed the role of the E-cadherin expression in brain morphogenesis using organ cultures, and found that this molecule is involved in local pattern formation of brain tissues.

Materials and methods

Mouse embryos

Mouse embryos were derived from matings of inbred ICR mice. The midpoint of the dark period was designated as E0 and more precise developmental stages of the collected embryos were determined according to Theiler (1972).

Antibodies

A rabbit antiserum to mouse E-cadherin (Nagafuchi et al., 1987) was used for inhibiting this molecule, and also for immunohistochemistry. The monoclonal antibodies ECCD-2 (Shirayoshi et al., 1986) and ECCD-1 (Yoshida-Noro et al., 1984) to E-cadherin were also used. A rabbit antiserum against the mouse *En-2/TrpE* fusion protein, called αEnhb-1 (Davis et al., 1991), which recognizes both *En-1* and *En-2* products, was kindly provided from Dr Alexandra Joyner.

Whole-mount antibody staining of embryonic brains

Brains were isolated from embryos of various developmental stages, freed from mesenchymal tissues, pia mater and cranial ganglia, and washed in ice-cold Hanks' solution to remove blood cells. When necessary, the isolated brains were cut in half sagittally. These were fixed with 4% paraformaldehyde for 1 hour at 4°C and subsequently with methanol for 20 minutes at −20°C. They were rehydrated with Hepes-buffered (pH 7.4) saline containing 1 mM $CaCl_2$ and 0.1% Triton-X (HBS-T), then incubated in 1% BSA/HBS-T containing 0.8% hydrogen peroxide for blocking non-specific binding of antibodies and for inactivating endogenous peroxidase for 6 hours at 4°C. The samples were washed several times with HBS-T to remove remaining hydrogen peroxide and incubated with the rabbit anti-E-cadherin antiserum diluted 1: 1,000 in 1% BSA/HBS-T for 6 hours at room temperature. After washing, these were incubated with HRP-conjugated anti-rabbit IgG antibody (Cappel) for 6 hours, and then with 0.5 mg/ml diaminobenzidine and 0.03% hydrogen peroxide in a Tris-buffered (pH 7.6) saline containing 1 mM $CaCl_2$ and 0.1% Triton-X. These samples were cleared with a 1 : 2 mixture of benzyl alcohol and benzyl benzoate (BABB; Dent et al., 1989). For staining of *En*, the method of Davis et al. (1991) was used.

Immunohistochemistry and cytochemistry

Immunostaining of cryosections or monolayer cell cultures was carried out as described before (Hatta et al., 1987). Briefly, embryos were fixed with 4% paraformaldehyde in Hanks' solution for 2-6 hours at 4°C, embedded in Tissue-Tek OCT compound (Miles Scientific) and sectioned by a cryostat. 10 μm sections were collected on gelatin-chrome alum-coated glass slides. Cultured cells were prepared on cover slips and fixed with paraformaldehyde as described below. These samples were post-fixed with methanol for 20 minutes at −20°C, rehydrated, incubated subsequently with 5% skim-milk, primary antibodies and fluorescein-labelled secondary antibodies, and finally mounted with a 9 : 1 mixture of glycerol and 1 mg/ml paraphenylenediamine. These samples were observed under a Zeiss Axiophot microscope.

Monolayer cell cultures

E10.5 brains containing the midbrain and anterior hindbrain were collected in Hepes-buffered (pH 7.4) Ca^{2+}- and Mg^{2+}-free saline (HCMF), and transferred into ice-cold HCMF containing 1 mM EDTA and 0.5% BSA. After a brief incubation, non-neural tissues surrounding the neural tube, especially the epidermis, were carefully removed using a pair of fine forceps and these purified brain fragments were digested with 0.25% trypsin in HCMF at 37°C for 60 minutes under a continuous rotation. When necessary, 20 units/ml DNAase I and 1 mM $MgCl_2$ were added to digest DNA released from lysed cells. The trypsinized tissues were thoroughly dissociated into single cells by repetitive pipettings and plated onto 24 mm-square cover slips preincubated with 50 μg/ml mouse laminin (Iwaki Glass) in HCMF at 4°C for 12 hours, which were placed in 35 mm diameter dishes. The density of cells was 2×10^6 cells/dish. Cells were cultured in a 1 : 1 mixture of Dulbecco-modified Eagle's MEM and Ham's F12 medium supplemented with 10% fetal bovine serum (DH10) for 3 days and fixed with 4% paraformaldehyde in Hanks' solution for 15 minutes at 4°C.

Organ culture of embryonic brain fragments

E9.5 brains containing dien-, mesen-, meten- and myelencephalon were isolated, and cut along the dorsal midline so as to be flattened, then placed on 13 mm diameter Nuclepore filters with 8.0 μm pores (Costar, SN110414), setting the outside of the neural tube down. These filters were floated on the surface of culture medium that was a 1 : 1 mixture of DMEM and Ham's F12 medium supplemented with 10% heat-inactivated fetal bovine serum, and incubated for 4 days. When necessary, antibodies to be tested were added to the medium.

Measurement of brain explant growth

Photomicrographs of the explants were taken and the maximum distance between the lateral edges in the midbrain region was measured on the films for each explant. The data was processed using the Stat View™ II software (Abacus Concepts, Inc.).

Results

Expression of E-cadherin in developing brains

We studied E-cadherin expression in early brains by whole-mount immunostaining. As previously reported, E-cadherin is expressed in the ectoderm in early embryos, but it disappears from the part of the ectoderm that is differentiating into the neural plate (Nose and Takeichi, 1986). After the closure of the neural tube, however, E-cadherin reappeared in some regions of mesencephalon (midbrain) and diencephalon at around embryonic day 9.5 (E9.5; Fig. 1A). By E12, these E-cadherin-positive areas became more distinct, displaying characteristic patchy patterns (Fig. 1B, C). These areas included the posterior end of mesencephalon, some areas beside the ventral midline in the mesencephalic flexure, and parts of the lateral and ventral diencephalon. At E12, cerebellar rudiments in metencephalon (anterior hindbrain) began to express E-cadherin (region 6 in Fig. 1C) and its expression level increased at E13.5 (Fig. 1D). These E-cadherin-positive areas were more clearly seen in opened neural tubes (Fig. 1G). Such samples also showed that, from cerebellar rudiments, E-cadherin-positive axon-

like fibers grow out towards the ventral midline (Fig. 1H). Similar axonal outgrowth was observed in some of other E-cadherin-positive areas, such as region 3 in Fig. 1C. In addition to these areas, the dorsal midline in mesencephalon expressed E-cadherin after E12, showing double streaks (Fig. 1F). These streaks bifurcated when they entered into diencephalon. At around E14, E-cadherin began to disappear from cerebellar primordia as well as from other regions except at the dorsal midline which still expressed this molecule at E15 (Fig. 1E).

Immunofluorescence staining for E-cadherin on sections of embryonic brains revealed the following profiles of the distribution of this molecule. At E8-E8.5, when the neural plate was still open, E-cadherin expression was almost negative in this tissue, although faint signals were detected (Fig. 2A). At E10-12, E-cadherin expression was now obvious in various parts of the neural tube and the E-cadherin-positive areas were demarcated from E-cadherin-negative areas with sharp boundaries (Fig. 2B). In those areas, cells with various morphologies, round or fibrous, stained for E-cadherin and were distributed from the top to bottom in the neuroepithelial layer, although the ventricular zone tended

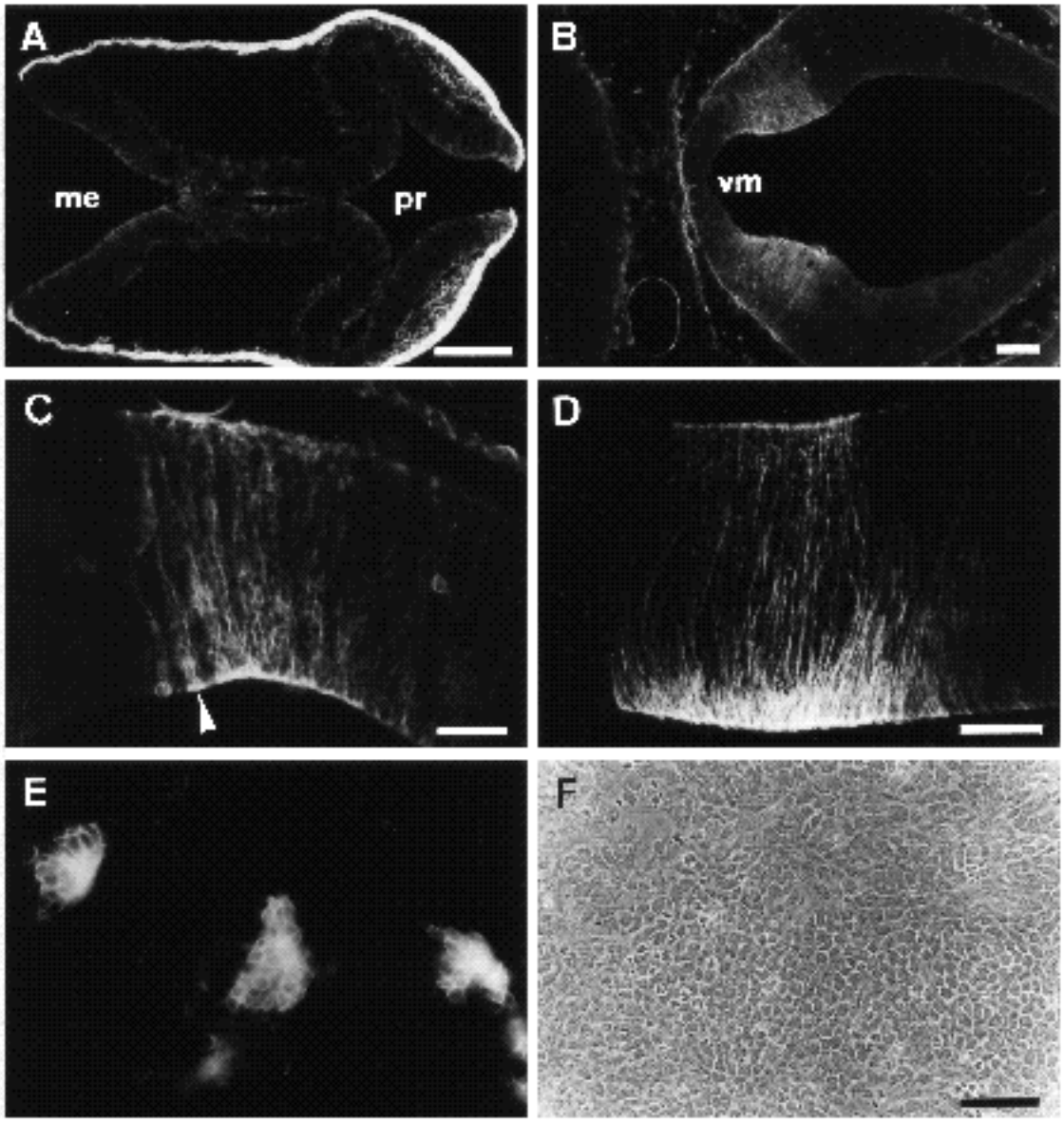

Fig. 2. Immunohistochemistry for E-cadherin on sections of embryonic brains. (A) A transverse section of an E8.5 embryo at the head region. The neural plate shows only a faint expression of E-cadherin, while the overlying ectoderm shows strong expression. (B) A cross section of an E12 embryo at the ventral diencephalon containing the region 5 in Fig. 1C. E-cadherin is expressed in restricted regions of diencephalon (hypothalamus). The ventral side is on the left. (C) A cross section of E12 brain at the lateral midbrain. Note that E-cadherin-positive cells form radially arranged clusters, (arrowhead). (D) A cross section of E12.5 brain at the diencephalon-mesencephalon border. The ventricular zone is on the bottom in C and D. (E) A confluent monolayer culture of cells derived from E10.5 midbrain and hindbrain, incubated for 3 days and stained for E-cadherin. (F) A phase-contrast micrograph of E. me, mesencephalon; pr, prosencephalon; vm, ventral midline. Scale bars, 100 μm for A, B and D; 50 μm for C, E and F.

to be more intensely stained (Fig. 2C). Closer examinations of these samples showed that E-cadherin-positive cells were radially clustered along fibrous structures; such pattern was most clearly seen at the peripheries of E-cadherin-positive regions, where E-cadherin-positive and -negative clusters were aligned side by side (Fig. 2C). At more advanced stages, fibrous structures were more conspicuous. At these stages, E-cadherin-positive cell bodies were restricted to the ventricular zone, while the fibrous structures reached the outer surface of the neural tube (Fig. 2D).

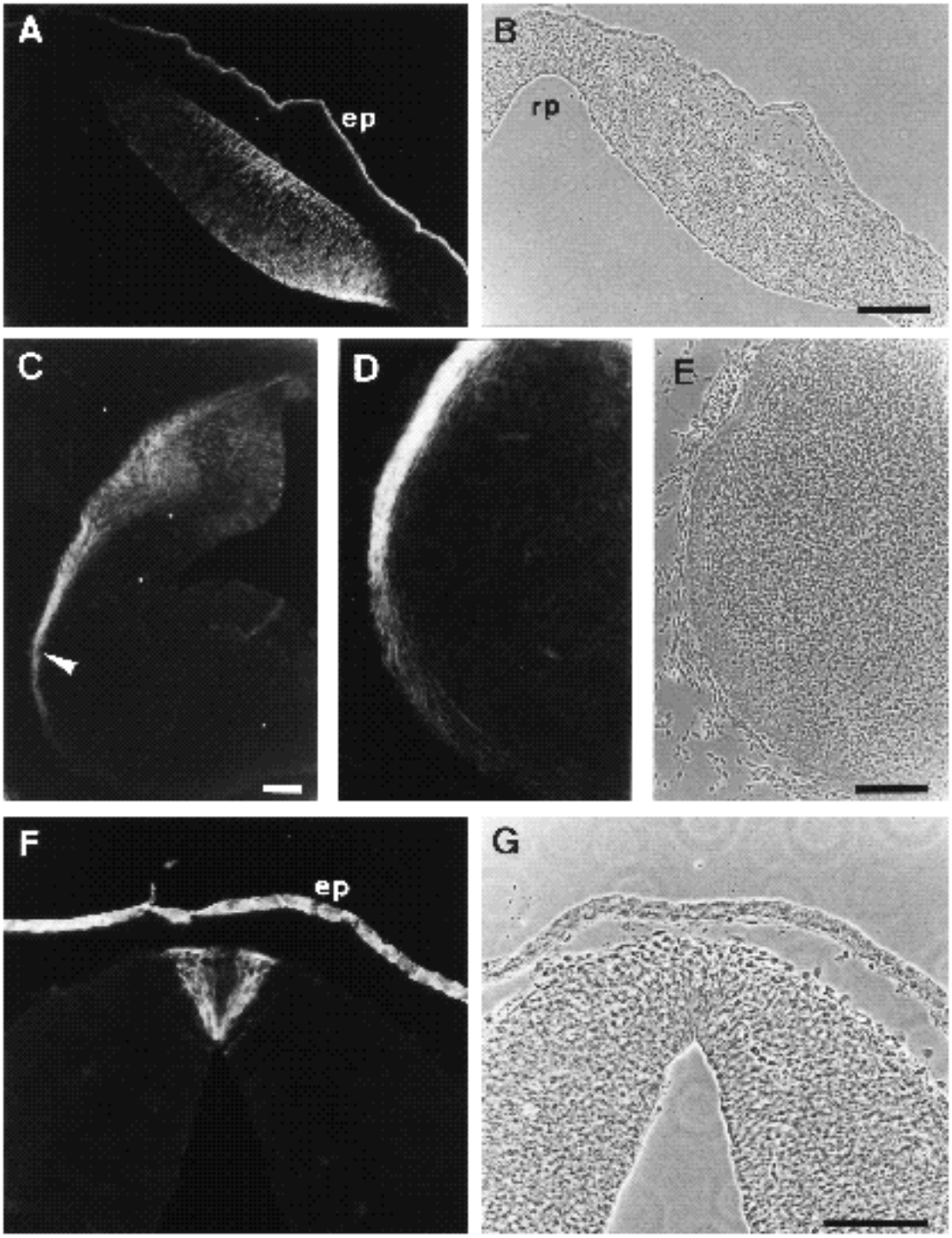

Fig. 3. Expression of E-cadherin in a cerebellar rudiment and the dorsal midline. (A) A transverse section of E12.5 cerebellar rudiment stained for E-cadherin. (B) A phase-contrast micrograph of A. (C) A region more posterior to A. (D) A higher magnification of the ventrolateral margin of the anterior hindbrain, which is indicated by arrowhead in C. (E) A phase-contrast micrograph of D. (F) A section of the dorsal region of E13.5 midbrain stained for E-cadherin. (G) A phase-contrast micrograph of a section adjacent to F. V-shaped E-cadherin-positive columns are seen in the dorsal midline. ep, epidermis; rp, roof plate. Scale bars, 100 μm.

In sections of metencephalon at E12.5, cerebellar primordia stained for E-cadherin in the pattern similar to that described above (Fig. 3A). From these regions, E-cadherin-positive fibers extended to the marginal zone and they migrated towards the ventral midline (Fig. 3C, D). These fibers probably correspond to the ones observed in the whole-mount samples (Fig. 1H). In the roof plate in mesencephalon, two E-cadherin-positive columns were observed (Fig. 3F, G), consistent with the results of whole-mount staining (Fig. 1F).

The same immunostaining patterns were obtained when the above tissues were stained with the monoclonal antibody ECCD-2 to E-cadherin, although its staining intensity was generally weaker than that of the polyclonal antibody.

Behavior of E-cadherin-expressing cells in vitro

E10.5 brains from which the forebrain and posterior hindbrain had been removed were completely dissociated into single cells. These cells were plated on dishes to produce monolayer cultures with high cell densities. 1-3 days after plating, the cultures were immunostained for E-cadherin to localize cells expressing this molecule. E-cadherin-positive cells always formed clusters, which were sharply segregated from adjacent E-cadherin-negative cells and they never randomly intermingled (Fig. 2E). Morphology of the E-cadherin-positive cells varied with the colonies; the majority of them were epithelioid, often displaying rosette-like cell arrangements and others comprised more differentiated neuron or glia-like cells.

Organ culture of embryonic brain fragments and the effect of anti-E-cadherin antibody on their growth

To study the role of E-cadherin in brain development, we established an organ culture system for embryonic brain fragments. Part of the neural tube, which consists of the entire mesencephalon and the anterior hindbrain, was isolated from a 9.5-day embryo. The neural tube was opened along the dorsal midline, placed on a Nuclepore filter (Fig. 4A) and cultured for 4 days. During the incubation, the overall size of the explants increased dramatically (Fig. 4B-D). When these explants were stained for E-cadherin, the original pattern of the distribution of this molecule was found to be preserved, although it was slightly modified by culturing (Fig. 5A).

We, then, cultured the brain fragments in the presence of polyclonal antibodies to E-cadherin which are known to strongly inhibit E-cadherin activity. When these antibody-treated explants were stained for E-cadherin, this molecule was found to have disappeared from most regions of the explants, except the otic vesicles and the dorsal midline that corresponded to the lateral edges of the explants (Fig. 5B). The disappearance of E-cadherin is probably due to endocytosis of the antibody-E-cadherin complexes by cells. Otic vesicle and dorsal midline cells, especially the former, express large amounts of E-cadherin proteins, so that endo-

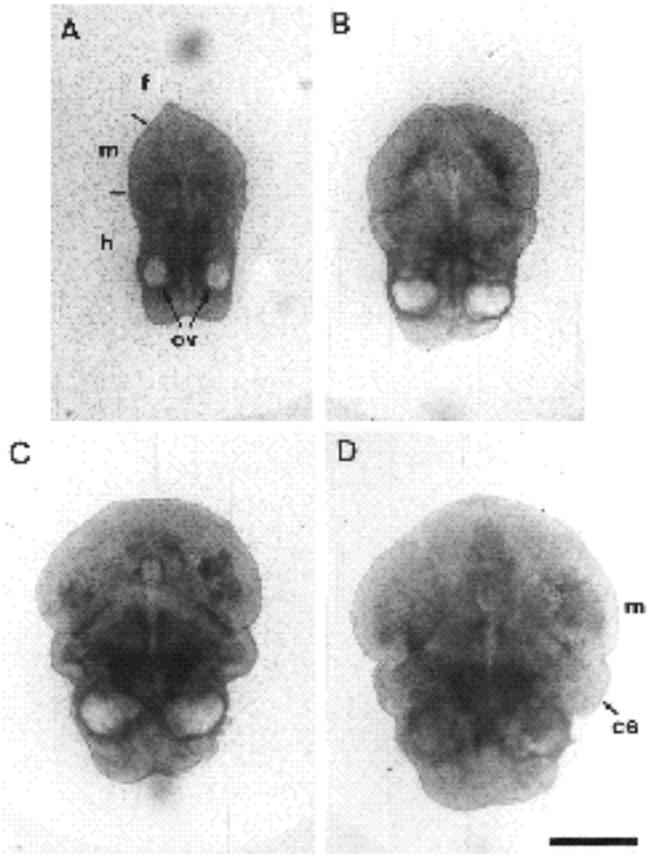

Fig. 4. Growth of a brain explant on Nuclepore filter. A fragment of E9.5 brain containing part of the forebrain, the entire midbrain and the anterior hindbrain was isolated, opened at the dorsal midline and explanted on a Nuclepore filter. (A) 0 days, (B) 1 day, (C) 2 days and (D) 3 days after incubation. A living sample was successively photographed. f, forebrain; m, midbrain; h, hindbrain; ov, otic vesicle; ce cerebellar rudiment. Scale bar, 1 mm.

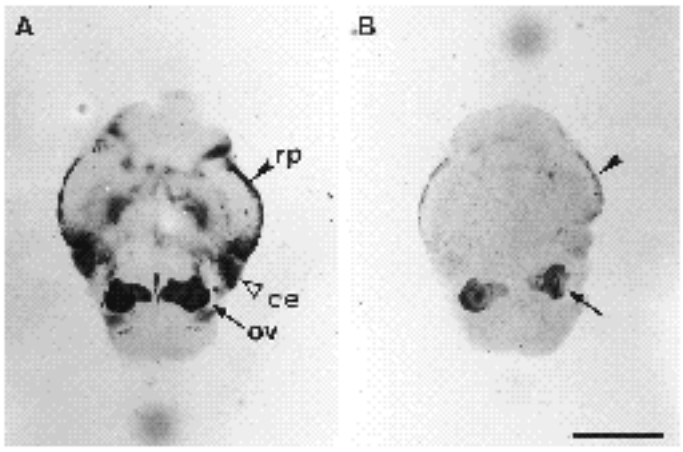

Fig. 5. Effect of anti-E-cadherin antibody on the expression of E-cadherin. (A) A control brain explant, prepared as in Fig. 4, cultured for 4 days in the presence of a preimmune rabbit serum diluted 1 : 1,000 and stained for E-cadherin. (B) An explant cultured in the presence of anti-E-cadherin antiserum diluted 1 : 1,000 for 4 days and stained for E-cadherin. Note that E-cadherin expression is preserved in A, but is absent in B, except in otic vesicles (arrow) and in the dorsal midline (roof plate) which was divided into the lateral edges of the explant (arrowhead). rp, roof plate; ce, cerebellar rudiment; ov, otic vesicle. Scale bar, 1 mm.

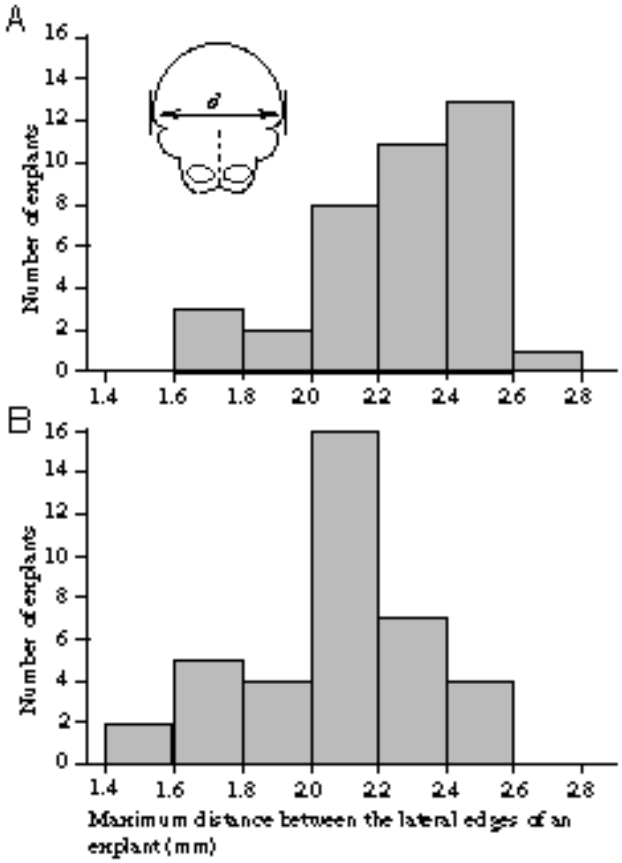

Fig. 6. Effect of anti-E-cadherin antibody on the growth of brain explants. E9.5 brain explants were prepared and cultured for 4 days in the absence (A) or presence (B) of anti-E-cadherin antiserum diluted 1 : 1,000, as in Fig. 5. The control cultures contained a preimmune serum diluted 1 : 1,000. The maximum distance between the lateral edges of each explant was measured and the frequency of the values obtained from 38 samples is shown in each figure. The difference between the mean values of control and experimental samples was tested by the two-sample *t*-test for the one tailed hypothesis. DF (degrees of freedom) = 74, $t = 2.98$ and $P<0.01$. The mean values±s.d. were 2.23±0.25 mm and 2.05±0.26 mm for the control and experimental samples, respectively.

cytosis probably could not have overcome the production of the proteins.

When brain explants cultured with and without the anti-E-cadherin antibody were compared, we noticed that the lateral expansion of mesencephalon, possibly also of other parts, tend to be slightly suppressed by the antibody (Fig. 5). This impression was confirmed by quantitative measurements of the distance between the lateral edges of mesencephalon in these explants (Fig. 6).

The above effects of anti-E-cadherin antibody were obtained with either purified antibodies (10 μg/ml) or the antiserum diluted 1:1,000 or with higher concentrations. The effective concentrations of the antibodies correlated with those necessary for inhibiting E-cadherin-mediated cell-cell adhesion (data not shown). The monoclonal antibody ECCD-2, which is known to tightly bind to E-cadherin without inhibiting its action (Shirayoshi et al., 1986), neither removed E-cadherin from the explants nor affected their growth. The monoclonal antibody ECCD-1, which blocks E-cadherin function by weakly binding to the antigens (Yoshida-Noro et al., 1984), also had no effect on either process. Mixtures of ECCD-2 and ECCD-1, however, showed the same effect as the polyclonal anti-E-cadherin antibody. These results suggest that the removal of E-cadherin from the cell surface is essential for complete blocking of its function in brain explants. Preimmune rabbit sera had no effects on these phenomena.

Effect of anti-E-cadherin antibody on En *expression*

The mouse *engrailed* homologue *En* is expressed in regions around the boundary between the midbrain and hindbrain (Davis et al., 1991), which include cerebellar primordia. Comparisons of E-cadherin and *En* expression in these regions showed that their distributions partly overlap (compare Fig. 7A and B for E12 brains, and Fig. 7C and E for cultured brains). We tested whether this *En* distribution pattern is affected when the explants were incubated with anti-E-cadherin antibody. The results showed that the *En* expression pattern was altered by this antibody treatment (Fig. 7C, D). The *En* belt at the midbrain-hindbrain boundary was a little thicker in the treated explants than in the controls and the curvature of this belt was reduced by the antibody treatment.

Discussion

E-cadherin (uvomorulin) is known to be important for epithelial cell-cell adhesions, but our previous (Takeichi et al., 1990; Shimamura et al., 1992) and present studies demonstrated that this molecule is also expressed in the nervous system. Its expression in embryonic brains was, however, local and transient. Most of the E-cadherin expression disappeared from the brain after E14, although it persisted in the roof plate area until later developmental stages. Thus, the major role of E-cadherin expression in brain morphogenesis, if any, should be restricted to early development.

Cell types expressing E-cadherin could not be identified biochemically but only morphologically in the present study, because we failed in finding appropriate cell type-specific markers whose expressions overlap with that of E-cadherin. For example, axon-like E-cadherin-positive fibers growing out from cerebellar primordia did not react with antibodies to the 200×10^3 M_r subunit of neurofilaments, but this did not necessarily rule out the possibility that they are axons. It seems that E-cadherin first appears in undifferentiated neural precursor cells, as many of the E-cadherin-positive cells formed rosette-like or epithelioid colonies in monolayer cultures, which are characteristic of ventricular neuroepithelial cells. At later developmental stages, they probably differentiate into various cell types, which include neurons and radial glia, as inferred from the morphology of E-cadherin-positive cells in more differentiated brains (for example, Figs 1H, 2D).

We show in the present study that E-cadherin-positive cells form isolated clusters in high density cultures of brain cells and they do not intermingle with surrounding E-cadherin-negative cells. These clusters were probably gener-

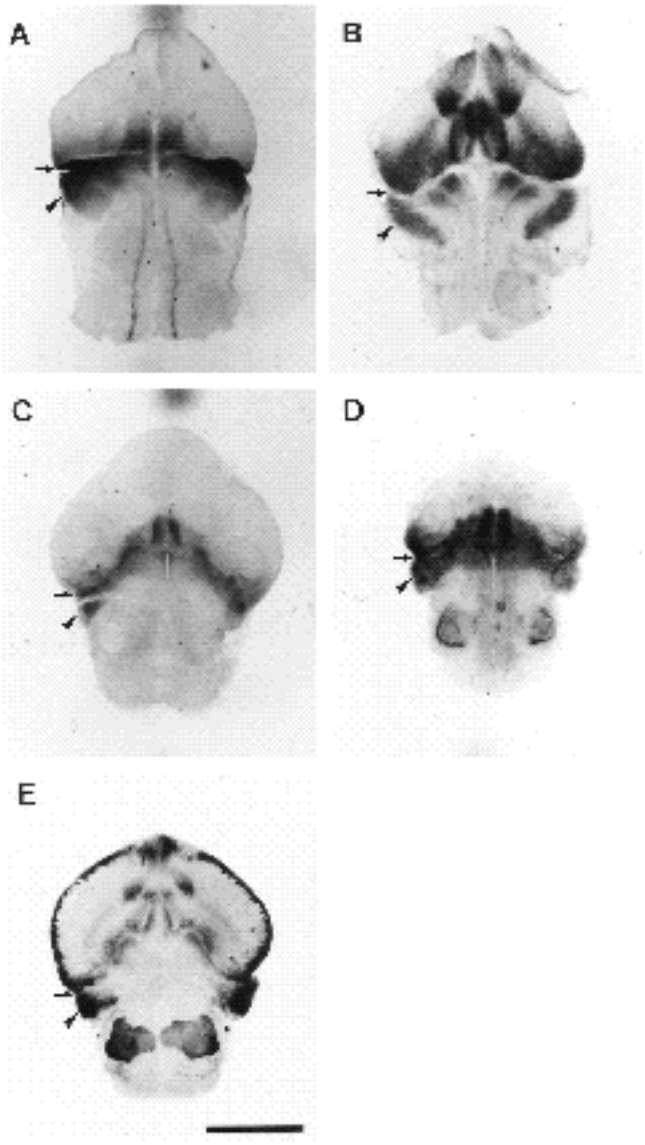

Fig. 7. Comparisons of the expression patterns of E-cadherin and *En*, and the effect of anti E-cadherin antibody treatment on *En* expression. (A,B) Opened fragments of E12 brain were stained for *En* (A) and E-cadherin (B). (C,D) Brain explants were cultured for 4 days in the absence (C) or presence (D) of anti-E-cadherin antiserum and stained for *En*. (E) A control sample prepared as in C and stained for E-cadherin. Arrows indicate the midbrain-hindbrain boundaries and arrowheads indicate cerebellar rudiments. Differences in the morphology of the explants between A and B are due to the use of different fixatives: *En* protein was immunostained after fixation with a 4 : 1 mixture of methanol and dimethylsulfoxide, as recommended by Davis et al. (1991), and this fixation caused shrinking of tissues in some samples such as the one in A. The samples for staining for E-cadherin were fixed with paraformaldehyde, as this method gave best immunostaining results. Scale bar, 1 mm.

ated by two successive processes. As we found that E-cadherin-positive cells are already clustered within one day of culture, the initial clustering of the cells might be brought about by the classical sorting out mechanism. Thereafter, each cluster must grow by cell proliferation. Homophilic interactions between E-cadherin molecules likely play a role in the initial sorting of the cells and also in preventing them from freely migrating out of the colonies once formed, although other cadherins or adhesion molecules might also be involved in such process.

A similar mechanism might operate also in vivo, and a role for the local expression of E-cadherin in embryonic brains could be to establish and maintain compartments of particular cell groups in the neural tube. Developing neural cells radially migrate in the neuroepithelial layers. During these movements, cells derived from the same precursor tend to stay in a single cluster, although some of them eventually leave the cluster in horizontal directions after differentiation (Gray and Sanes, 1991). There should be some mechanisms to regulate such controlled cell movements. In the present study, we observed an interesting distribution pattern of the E-cadherin expressing cells in vivo. These cells were not evenly distributed in the E-cadherin-positive areas, but were grouped into clusters that were radially arranged in the nerual tube (Fig. 2C). Cells constituting each cluster were possibly derived from a single precursor, and E-cadherin might participate in grouping these cells of the same clonal origin. This possibility could be tested by examining whether anti-E-cadherin antibodies can perturb the distribution of these cells. However, such experiments would not be technically easy without some specific markers of the E-cadherin-positive cells, since E-cadherin itself disappears during the antibody treatment.

A striking finding in the present study is that anti-E-cadherin antibody affected the overall morphology of developing brains in vitro. The lateral expansion of brain explants was retarded and the pattern of *En* distribution was altered by the antibody treatment. *En* is expressed in the boundary of midbrain and hindbrain, and its expression pattern partly overlapped with that of E-cadherin. Therefore, *En* served as a positional marker for the cells located in these regions. The alteration of *En* expression pattern implies that the arrangement of cells was modified in these regions.

Previous studies demonstrated that the treatment of tissues with antibodies to cadherins causes disorganization of cell arrangements. Epithelial tubes are crushed (Hirai et al., 1989a) and early neural retinas are dissociated into cell clusters (Matsunaga et al., 1988), when these tissues were incubated with the antibodies that can inhibit the cadherins expressed by them. Local overexpression or ectopic expression of N-cadherin in *Xenopus* embryos also affected tissue morphology (Detrik et al., 1990; Fujimori et al., 1990). These observations suggest that the amount or distribution of cadherins in tissues are crucial for determining their overall morphology. It is thus likely that abnormal morphogenesis of the brain explants observed in the presence of anti-E-cadherin antibody is due to some perturbation of cell arrangements. Alternatively, some specific functions of E-cadherin-positive cells might have been lost by the antibody treatment, affecting morphogenesis or growth of the explants. In any case, the present observations suggest that the characteristic distribution pattern of E-cadherin in embryonic brain is important in determining local morphology of this organ.

Morphogenesis of the brain is assumed to be regulated

by a complex gene cascade. *Wnt-1* is known to be involved in such a cascade. This gene encodes a secretory protein and is expressed in local regions of CNS, including parts of the mesencephalon and the dorsal midline of brain ventricles (Wilkinson et al., 1987). It thus appears that the *Wnt-1*-positive regions at least partly overlap with E-cadherin-positive regions in the brain of 9.5- to 13.5-day embryos. This possible overlapping, together with a correlation between *En* and E-cadherin expression, suggests that there might be some functional links among the three gene products.

Drosophila homologues of *Wnt-1* and *En* are *wingless* and *engrailed*, respectively (Rijsewijk et al., 1987; Joyner et al., 1985; Joyner and Martin, 1987). These *Drosophila* genes are indeed functionally linked to each other; that is, the *wingless* gene product is crucial for the expression of *engrailed* (DiNardo et al., 1988). Recently, the *Drosophila* segment polarity gene *armadillo* was found to encode a protein resembling β-catenin which is known to be a cadherin-associated protein in vertebrates (McCrea et al., 1991). Interestingly, the phenotype of the *armadillo* mutant is similar to that of the *wingless* mutant (Peifer et al., 1991). These findings suggest that the putative *Drosophila* cadherin adhesion system might be involved in a signaling pathway of *wingless.* Assuming that a similar mechanism operates in vertebrates, the correlations found among the expression patterns of E-cadherin, *Wnt-1* and *En* may not be coincidental. To determine a possible link among these molecules, therefore, should be an intriguing future subject for investigation.

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Fig. 1. Whole-mount immunostaining for E-cadherin in developing mouse brains. (A) E9.5, (B) E11, (C) E12, (D) E13.5 and (E) E14.5. Brains were cut sagittally in half and stained for E-cadherin. B is a view from the outer surface of neural tube, while A, C, D and E are views from the ventricular side. Arrows indicate E-cadherin-positive regions. Stainings on the forebrain are not specific for E-cadherin. (F) A dorsal view of E12.5 brain stained for E-cadherin. The dorsal midline in mesencephalon is stained as double streaks, which bifurcate in diencephalon, as shown by arrowheads. (G) A fragment of E12 brain, comprising part of diencephalon, the midbrain and the anterior hindbrain, which was opened at the dorsal midline and cleared with BABB after E-cadherin staining. (H) A cerebellar rudiment and adjacent areas in E13.5 brain prepared as in G. Note that E-cadherin-positive fibers grow out from the rudiment to the ventral midline. Arrowheads in G and H indicate E-cadherin-positive cerebellar rudiments. Numbers indicate the corresponding regions in different panels. ce, cerebellar rudiment; me, mesencephalon; di, diencephalon; te, telencephalon; my, myelencephalon; vm, ventral midline. Scale bars, 500 μm for A-G; 100 μm for H.