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Sonic hedgehog: a common signal for ventral patterning along the rostrocaudal axis of the neural tube

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ABSTRACT The vertebrate hedgehog-related gene, *sonic hedgehog,* is expressed in ventral domains along the entire rostrocaudal length of the neural tube, including the forebrain. Shh induces the differentiation of ventral neuronal cell types in explants derived from prospective forebrain regions of the neural plate. Neurons induced in explants derived from both diencephalic and telencephalic levels of the neural plate express the LIM homeodomain protein Islet-1, but these neurons possess distinct identities that match those of the ventral neurons normally generated in these two subdivisions of the forebrain. These results, together with other studies of neuronal differentiation at caudal levels of the neural tube, suggest that a single inducing molecule, Shh, mediates the induction of distinct ventral neuronal cell types along the entire rostrocaudal extent of the embryonic central nervous system.

KEY WORDS: *Islet-1, Sonic hedgehog, forebrain, ventral patterning*

Introduction

In vertebrate embryos, the patterning of the nervous system is initiated by inductive signals that act over short distances to direct the fate of neural progenitor cells. The complex pattern of cell types generated within the neural tube is thought to involve the action of signals that impose regional character on cells at different rostrocaudal positions within the neural plate (Doniach *et al.,* 1992; Ruiz i Altaba, 1992; Papalopulu, 1994) and that define the identity of cells along the dorsoventral axis of the neural tube (Jessell and Dodd, 1992; Basler *et al.,* 1993; Smith, 1993). Thus, the fate of neural progenitor cells depends on their position along the rostrocaudal and dorsoventral axes of the neural tube.

The mechanisms that control the differentiation of cell types along the dorsoventral axis of the neural tube have been examined in great detail at caudal levels of the neuraxis. In the spinal cord, the differentiation of ventral cell types is initiated by signals transmitted from axial mesodermal cells of the notochord to overlying neural plate cells, inducing the differentiation of floor plate cells at the ventral midline and motor neurons more laterally within the neural tube (van Straaten *et al.,* 1988; Placzek *et al.,* 1990, 1991; Yamada *et al.,* 1991, 1993; Goulding *et al.,* 1993). At later stages, similar or identical signalling properties are acquired by floor plate cells (Yamada *et al.,* 1991; Placzek *et al.,* 1993). The specific identity of the ventral neuronal cell types that are generated in response to notochord- and floor plate-derived signals, however, appears to be defined by the position of origin of neuronal progenitor cells along the rostrocaudal axis. For example, serotonergic neurons are induced by midline-derived signals at the level of the rostral rhombencephalon (Yamada *et al.,* 1991) whereas dopaminergic neurons are induced at the level of the mesencephalon (Hynes *et al.,* 1995).

At caudal levels of the neuraxis, a vertebrate homolog of the secreted glycoprotein encoded by the Drosophila gene *hedgehog* (Nüsslein-Volhard and Wieschaus, 1980; Lee *et al.,* 1992), *sonic hedgehog (shh),* has been implicated in the induction of ventral cell types. *shh* is expressed by the notochord and floor plate at the time that these two cell groups exhibit their inductive activities (Krauss *et al.,* 1993; Echelard *et al.,* 1993; Riddle *et al.,* 1993; Chang *et al.,* 1994; Roelink *et al.,* 1994). Furthermore, exposure of neural plate explants to Shh leads to the differentiation of motor neurons in addition to floor plate cells (Roelink *et al.,* 1994), suggesting that Shh participates in the induction of ventral neurons at caudal levels of the neuraxis.

At most levels of the embryonic forebrain, the notochord and floor plate are absent (Kingsbury, 1930; Puelles and Rubenstein, 1993) and neither the identity nor the source of inductive signals that trigger the differentiation of ventral neurons have been established. *shh* is expressed by cells at the ventral midline of the embryonic forebrain (Echelard *et al.,* 1993; Krauss *et al.,* 1993; Chang *et al.,* 1994; Roelink *et al.,* 1994), raising the possibility that this gene participates in the specifications of neuronal identity within the forebrain as well as at more caudal levels of the neuraxis.

Abbreviations used in this paper: shh, sonic hedgehog; HH stage, Hamburger and Hamilton stage.

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The results described in this review show that Shh induces ventral neuronal cell types normally found in the forebrain in addition to inducing motor neurons at more caudal levels of the neural tube. These findings suggest that a single inducing molecule, Shh, is responsible for inducing ventral neuronal cell types along the entire rostrocaudal extent of the neuraxis. They also indicate that the repertoire of ventral neuronal cell types that can be induced by Shh is defined by an earlier restriction in the rostrocaudal character of cells within the neural plate (Ericson *et al.*, 1995).

Results

shh and Islet-1 occupy adjacent ventral domains in the embryonic CNS

At caudal levels of the neuraxis, the differentiation of motor neurons depends on inductive signals provided by the notochord and floor plate (Yamada *et al.*, 1991, 1993). The earliest marker of differentiating motor neurons is the LIM homeodomain protein Islet-1 (Karlsson *et al.*, 1990; Ericson *et al.*, 1992; Inoue *et al.*, 1993; Korzh *et al.*, 1993; Tsuchida *et al.*, 1994). Although motor neurons are absent from the forebrain, Islet-1 is expressed by ventral neurons in the adult forebrain (Thor *et al.*, 1991).

Comparison of the patterns of expression of Islet-1 and *shh* in the embryonic chick nervous system shows that each Islet-1$^+$ cell group abut the domain of expression of *shh* (Fig. 1; see Fig. 2Ai for a summary). In the spinal cord, rhombencephalon and mesencephalon, *shh* is expressed by floor plate cells at the ventral midline (Fig. 1B,F,G and data not shown) and Islet-1 is expressed by cells located lateral to the floor plate (Fig. 1B,F,G and data not shown). In the mid-diencephalon at the level of the infundibulum, *shh* is not expressed at the ventral midline but is located more laterally (Fig. 1A,D). Islet-1$^+$ cells are also excluded from the ventral midline but are located immediately lateral to the zone of *shh* expression (Fig 1D). In the rostral diencephalon, *shh* is expressed at the ventral midline of the neural tube and is restricted to the ventricular zone (Fig. 1E,H,I). Within this region, Islet-1$^+$ cells are also located at the midline, immediately adjacent to the domain of expression of *shh* (Fig. 1I). In the telencephalon, the zone of *shh* expression also spans the ventral midline of the neural tube (Fig. 1J,K). Islet-1$^+$ cells are also restricted ventrally and are intermingled with cells expressing *shh* (Fig 1K). These results indicate that Islet-1 expression defines ventral cell types at forebrain as well as at more caudal levels of the neural tube (Ericson *et al.*, 1995).

At all levels of the neuraxis, with the exception of the telencephalon, the expression of *shh* precedes the differentiation of Islet-1$^+$ cells. In the ventral telencephalon, however, expression of *shh* is coincident with the onset of Islet-1 expression.

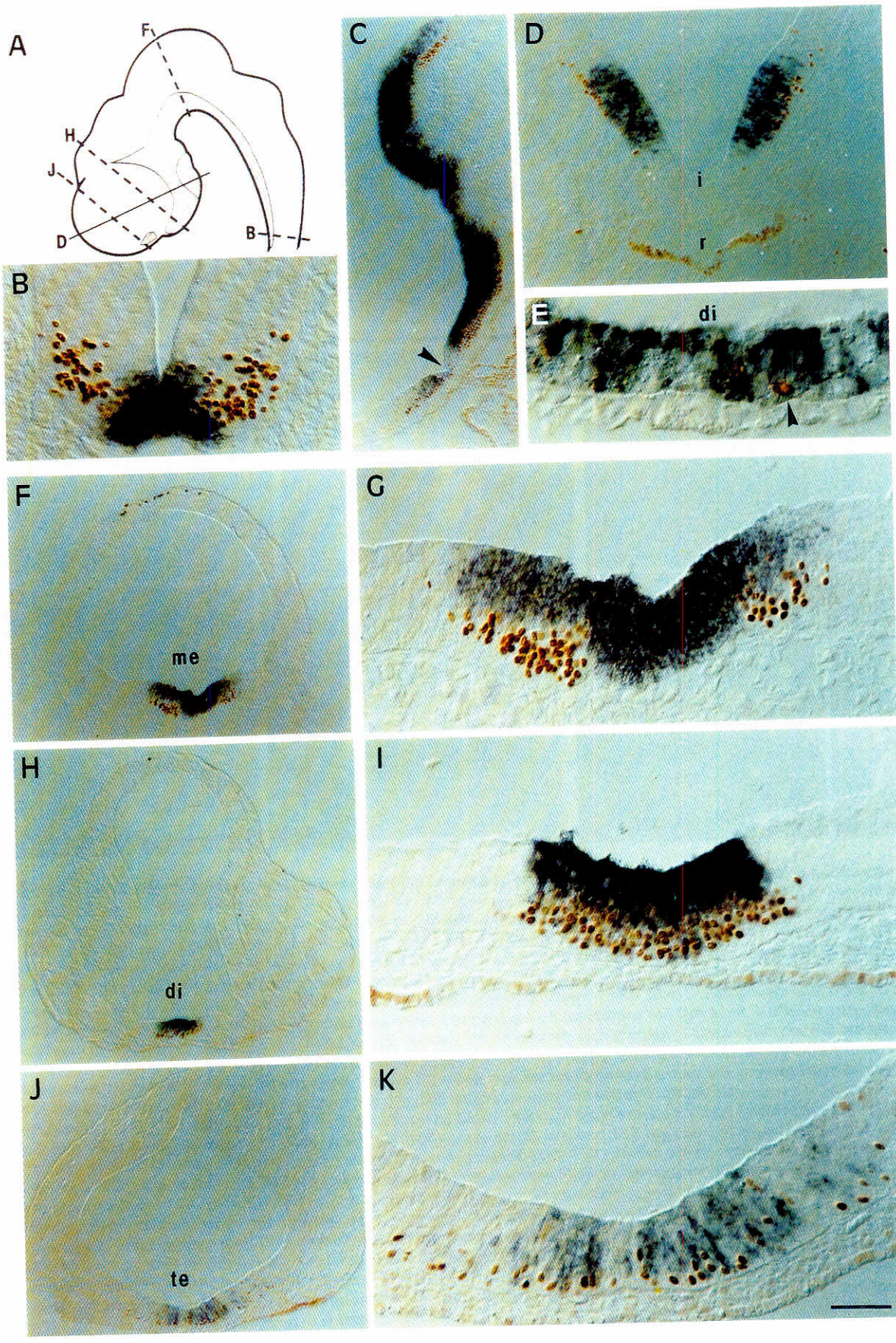
Cells that express Islet-1 at different axial levels are neurons with distinct identities

Double-label immunocytochemistry with antibodies directed against Islet-1 and the neuron-specific markers confirms the identity of the Islet-1$^+$ cells as neurons. Although all Islet-1$^+$ cells are neurons, however, their identities at different rostrocaudal positions are distinct.

In the rhombencephalon and mesencephalon, the Islet-1$^+$ neurons express the immunoglobulin-like surface protein SC1 (Fig. 2Aii, B and data not shown), in common with spinal motor neurons (Yamada *et al.*, 1991; Ericson *et al.*, 1992). Neither diencephalic nor telencephalic Islet-1$^+$ neurons express SC1 (Fig. 2C, see also Table 2).

In mouse embryos, the homeodomain-containing protein Nkx 2.1 is expressed at prospective diencephalic and telencephalic levels of the neural tube in a ventral domain that overlaps with that of *shh,* but the gene is not expressed at rhombencephalic or spinal cord levels (Lazzaro *et al.*, 1991; Price *et al.*, 1992; Rubenstein *et al.*, 1994). In chick embryos examined at HH stages 14-18 (Hamburger and Hamilton, 1951), antibodies directed against Nkx 2.1 label cells in a broad ventral domain of the mid and rostral diencephalon and telencephalon (Fig. 2Aiii,

Fig. 1. *shh* and Islet-1 are expressed in adjacent ventral domains in the embryonic chick central nervous system. (A) *Saggital view showing the domain of shh expression in the central nervous system of a HH stage 18/19 chick embryo (shaded area). The dashed lines indicate the axial levels and planes of the sections shown in panels* **B-K**. **(B-K)** *The domains of shh mRNA (blue-black) and Islet-1 (brown) express in adjacent domains of the ventral CNS.* **(B)** *A transverse section through the caudal rhombencephalon showing shh expression at the ventral midline in the floor plate and Islet-1 expression laterally, in motor neurons.* **(C)** *A saggital section of the neural tube showing shh and Islet-1 expression in the ventral mesencephalon, diencephalon and telencephalon. In the mesencephalon and rostral diencephalon, cells that express Islet-1 are located adjacent to the ventral domain of expression of shh. shh expression is detected in the basal telencephalon, rostral to the optic chiasm (arrowhead) and here, Islet-1 cells are found ventral and rostral to the domain of shh expression. Note that there is a region at the rostral-most tip of the ventral diencephalon, abutting the optic chiasm, that does not express shh.* **(D)** *A transverse section through the mid-diencephalon at the level of infundibulum (i). Cells that express shh form two bilateral stripes. Cells that express Islet-1 are located at the lateral edge of the domain of shh expression. Islet-1$^+$ cells are absent from the ventral midline at the level of the infundibulum. Cells at the ventral region of Rathke's pouch (r) express Islet-1.* **(E)** *In the rostral diencephalon at HH stage 13, cells that express Islet-1 are interspersed with cells that express shh. The double labeling method does not clarify whether any cells coexpress shh and Islet-1 at this stage.* **(F)** *A transverse section through the mesencephalon showing ventral midline expression of shh and Islet-1. At this axial level, a small number of Islet-1$^+$ sensory neurons can also be detected dorsally, in the trigeminal mesencephalic nucleus.* **(G)** *Higher magnification of* **(F)** *showing that the domain of shh expression expands lateral to the midline and that Islet-1 cells are located lateral to the midline domain of shh expression.* **(H)** *A transverse section at the level of the rostral diencephalon showing ventral midline expression of shh and Islet-1.* **(I)** *Higher magnification of* **(H)** *showing the ventral midline of the rostral diencephalon. Both shh and Islet-1 are expressed at the midline of the rostral diencephalon. shh is expressed in the ventricular zone whereas Islet-1$^+$ cells are located basally.* **(J)** *A transverse section at the level of the caudal telencephalon showing shh and Islet-1 cells in the floor of the telencephalon.* **(K)** *Higher magnification of* **(J)**. *In the ventral telencephalon cells that express shh and Islet-1 are more dispersed then at caudal regions of the ventral CNS. The lack of shh expression by cells at the ventral midline suture of the telencephalon is a consistent observation. Whole-mount in situ hybridization was performed using a chick Islet-2 probe (Tsuchida et al., 1994). Chick Islet-2 mRNA was not expressed at rhombencephalic, mesencephalic, diencephalic or telencephalic levels, indicating that immunoreactivity detected with the Islet-1 antisera corresponds to the Islet-1 protein (data not shown). Abbreviations: i, infundibulum; di, diencephalon; me, mesencephalon; te, telencephalon. Scale bar: B,G,I,K, 50 μm; C,F,H,J , 200 μm; D, 100 μm; E, 25 μm. Reproduced with permission from Cell.*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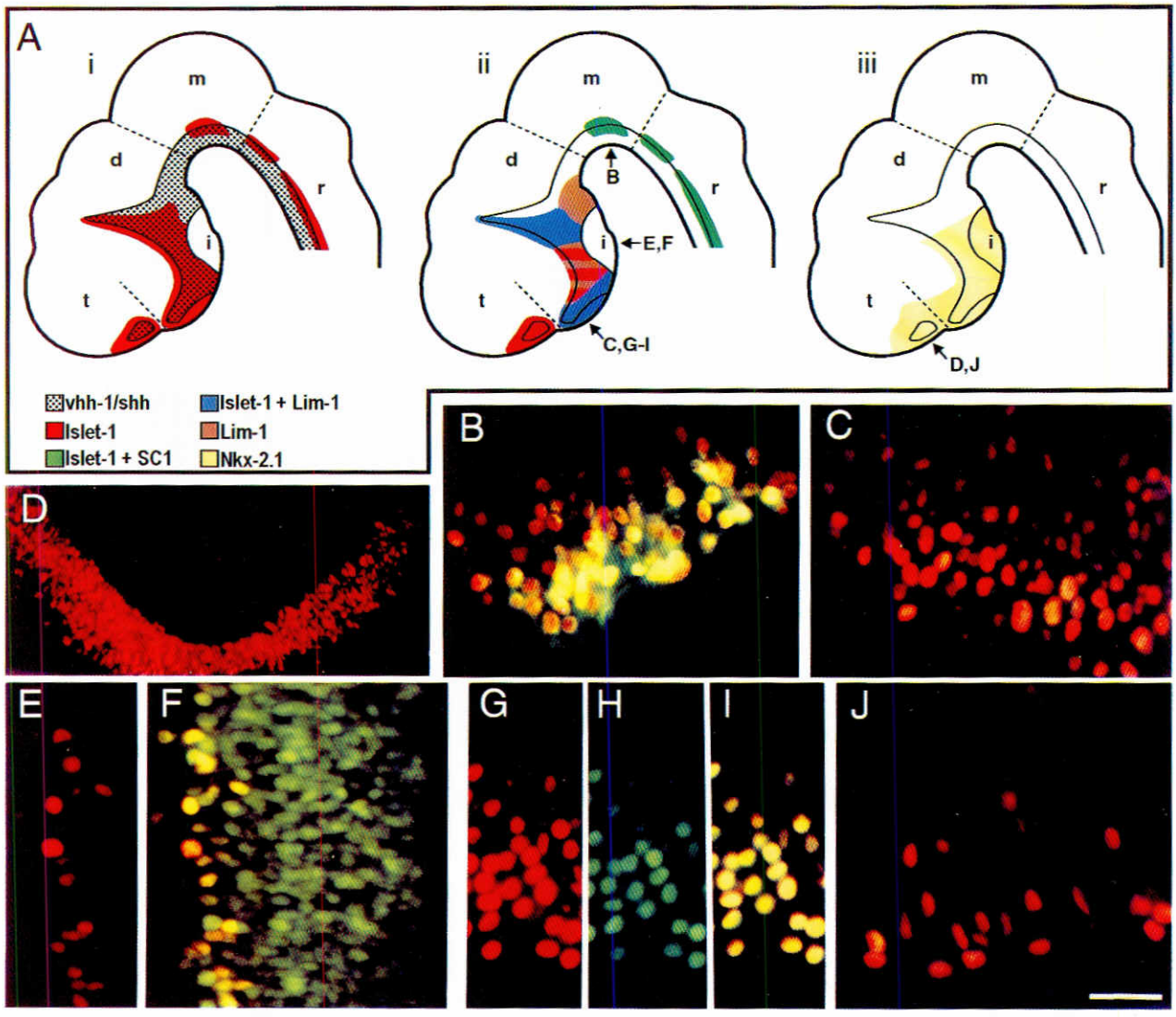

Fig. 2. Islet-1 expression defines distinct populations of ventral neurons at different rostrocaudal levels of the neuraxis. (A) *Diagram of a sagittal section of the neural tube of a HH stage 18/19 chick embryo showing the domains of expression of cell type markers. i) Summary diagram of the domains of expression shh (stippled) and Islet-1 (red) derived from whole-mount labeling shown in Fig. 1. ii) Summary diagram showing the coexpression of markers in Islet-1⁺ neurons. In the rhombencephalon (r) and mesencephalon (m), ventral Islet-1⁺ neurons coexpress the surface immunoglobulin protein SC1 (green domain). In the ventral diencephalon, Islet-1⁺ neurons are absent from the most caudal region, although Lim-1⁺ cells (brown) are expressed. In the region of the mid-diencephalon, rostral to the zona limitans interthalamica (Puelles et al., 1987), and also at the ventral midline of the rostral diencephalon, most Islet-1⁺ neurons coexpress Lim-1 (blue domain). In the intervening region of the mid-diencephalon above the infundibulum (i), Islet-1 and Lim-1 are expressed in separate but intermingled neuronal populations (domain indicated by brown and red stripes). In the ventral telencephalon, Islet-1⁺ neurons (red domain) do not express SC1 or Lim-1. For simplicity, the domain of neuroepithelial Lim-1 expression that occupies the entire dorsoventral extent of the mid-diencephalon, rostral to the zona limitans interthalamica is not depicted in this diagram. iii) Summary diagram showing the ventral domain of expression of Nkx 2.1 protein. Small arrows indicate the plane of sections shown in panels* **B-J. (B)** *Ventral detail of a transverse section through the mesencephalon showing that motor neurons of oculomotor (III) nucleus coexpress Islet-1 (red) and SC1 (green). Oculomotor neurons are the most rostrally located group of Islet-1⁺ cells that coexpress SC1. Somatic visceral and brachial motor neurons at more caudal levels also express SC1 (see also Simon et al., 1994).* **(C)** *Ventral detail of a transverse section through the rostral diencephalon showing that Islet-1⁺ neurons do not express SC1. SC1-labeled axons in* ***C*** *derive from neurons located more rostrally that do not express Islet-1.* **(D)** *Detail of a transverse section through the ventral telencephalon showing expression of Nkx 2.1 in most cells.* **(E,F)** *Detail of a transverse section through the lateral region of the mid-diencephalon dorsal to the infundibulum (see Fig. 1D for a low power view) showing that virtually all undifferentiated neuroepithelial cells express Lim-1 at low levels* **(F)** *and that Islet-1⁺ neurons* **(E)** *(red) also coexpress Lim-1 (yellow cells in* **F***).* **(G,H,I)** *Ventral detail of a transverse section through the rostral diencephalon showing that Islet-1⁺ neurons* **(I)** *(red) express Lim-1* **(H)** *(green).* **(I)** *shows a double exposure of* **(G and H)** *to indicate the extent of overlap of labeled cells.* **(J)** *Ventral detail of a coronal section through the ventral telencephalon showing that Islet-1⁺ neurons do not express Lim-1, as shown by the absence of yellow cells in this double exposure of Islet-1 (rhodamine) and Lim-1 (FITC). Abbreviations: r, rhombencephalon; m, mesencephalon; d, diencephalon; t, telencephalon; i, infundibulum. The sections shown in* **(B-J)** *are from HH stage 18-19 embryos. Scale bar: B, 160 μm; C,E-I, 25 μm; D,J, 20 μm. Reproduced with permission from Cell.*

TABLE 1

INDUCTION OF Islet-1⁺ CELLS BY Shh IN NEURAL PLATE EXPLANTS

Region of neural plate	Transfection construct	(%) Islet-1⁺ explants	(%) Islet-1⁺ neurons/ explant	(%) Islet-1⁺ neurons that express Lim-1
Rhombencephalic:	Antisense */shh*	0 (49)	0	-
	Sense *shh*	57 (45)	39 (11)	-0 (16)
Diencephalic:	Antisense *shh*	0 (28)	0	-
	Sense *shh*	57 (30)	35 (9)	22 (11)
Telencephalic:	Antisense *shh*	0 (46)	0	0
	Sense *shh*	78 (42)	96 (7)	0 (15)

Neural plate explants isolated from telencephalic, diencephalic and rhombencephalic levels of HH stage 6 chick embryos were cultivated for 60-66 h in contact with COS cells transfected with a *shh* expression construct in sense or antisense orientation and the proportion of explants that express Islet-1 was determined by whole-mount immunohistochemistry. The percentage of Islet-1⁺ and Lim-1⁺ cells in Shh-induced explants was determined by sectioning explants and counting the number of labeled cells in individual sections. The total number of cells in explants was determined using DAPI nuclear staining. The number of explants analyzed is indicated in brackets.

D and data not shown). Nkx 2.1⁺ cells are not detected in the rhombencephalon or spinal cord (Fig. 2Aiii). The onset of expression of Nkx 2.1 in the diencephalon occurs at HH stage 9 and in the telencephalon at HH stage 13/14. The expression of Nkx 2.1 in the ventral forebrain is transient but when examined at HH stage 18, about 10% of Nkx 2.1⁺ cells coexpress Islet-1. Thus, the expression of Nkx 2.1 serves primarily as a marker of ventral forebrain cells but coexpression of Nkx 2.1 and Islet-1 distinguishes Islet-1⁺ neurons generated in the diencephalon and telencephalon from those found at more caudal levels.

In the embryonic mouse forebrain, *Lim-1* mRNA is restricted almost exclusively to the diencephalon (Barnes *et al.*, 1994; Fujii *et al.*, 1994). In chick embryos examined from HH stages 14-18, antibodies directed against Lim-1 (Tsuchida *et al.*, 1994) detect cells in the diencephalon in a pattern similar to that described for *Lim-1* mRNA in mouse (see Fig. 2Aii). At these stages Lim-1⁺ cells are not detected in the telencephalon (Fig 2A, and data not shown). In the mid-diencephalon, but not at other levels of the diencephalon, Lim-1 is expressed by neuroepithelial cells (Fig. 2Aii,F). At this axial level, Lim-1⁺ neurons are also present, moreover the majority of Islet-1⁺ neurons express Lim-1 (Fig. 2E,F). In the rostral diencephalon, Lim-1 is expressed in the same population of ventral midline neurons that express Islet-1 (Fig. 2G-I). In the intervening region of the diencephalon, Lim-1⁺ neurons are also present in a population distinct from, but intermingled with, Islet-1⁺ neurons (Fig. 2Aii). In the telencephalon, Islet-1⁺ neurons do not express Lim-1 (Fig. 2J). Thus, Lim-1 expression distinguishes diencephalic from telencephalic cells. Moreover, although Lim-1 is not a marker of all diencephalic Islet-1⁺ neurons, its coexpression with Islet-1 indicates the diencephalic origin of Islet-1⁺ forebrain neurons.

Shh induces Islet-1⁺ neurons in prospective forebrain regions of the neural plate

Based on a coarse fate map of the neural plate of HH stage 6 chick embryos explants from lateral regions of the neural plate were isolated at three different levels of the neuraxis: i) [T] in Figure 3A fated to give rise to the telencephalon; ii) [D] in Figure 3A fated to give rise to the diencephalon, and iii) [R] in Figure 3A fated to give rise to the rhombencephalon. When such neural plate explants were grown for 60-66 h *in vitro*, in the presence of COS cells transfected with antisense *shh* cDNA, cells in explants derived from all three axial levels express the neuronal marker ß-tubulin but Islet-1⁺ cells are not detected (Fig. 3B,C,F,G,J,K). In contrast, numerous Islet-1⁺ cells were induced in explants derived from each of the three axial levels of the neural plate when they are grown on COS cells transfected with sense *shh* cDNA (Fig. 3D,E,H,I,L,M; Table 1).

Islet-1⁺ neurons induced by Shh-1/shh have distinct axial identities

In rhombencephalic level explants that have been exposed to Shh, both Islet-1⁺/SC1 expressing motor neurons and SC1/FP1 expressing floor plate cells are induced. In diencephalic and telencephalic level explants, the Islet-1⁺ neurons induced by exposure to Shh do not coexpress SC1. Floor plate cells, defined by expression of FP1, are not detected in diencephalic or telencephalic level explants exposed to Shh. Neural plate explants do not express Nkx 2.1 when grown on COS cells transfected with antisense *shh* cDNA and Nkx 2.1⁺ cells are not detected in rhombencephalic level explants exposed to Shh whereas induced diencephalic and telencephalic level explants contain Nkx 2.1⁺ cells and after 48 h *in vitro* 70% of the Islet-1 cells coexpress Nkx 2.1. Lim-1⁺ cells are detected in rhombencephalic and diencephalic but not telencephalic level explants grown on COS cells transfected with antisense *shh* cDNA. These results are summarized in Table 2. In diencephalic level explants exposed to Shh, around 25% of Islet-1⁺ neurons

TABLE 2

MARKER EXPRESSION IN EXPLANTS DERIVED FROM DIFFERENT AXIAL LEVELS OF THE NEURAL PLATE

Region of neural plate	Transfection construct	Marker Expression Islet-1	SC1	Nkx 2.1	Lim-1
Rhombencephalic:	Antisense *shh*	-	-	-	++
	Sense *shh*	++	++	-	+
Diencephalic:	Antisense *shh*	-	-	-	++
	Sense *shh*	++	-	+	++
Telencephalic:	Antisense *vhh-1/shh*	-	-	-	-
	Sense *shh*	+++	-	+	-

Analysis of neural plate explants grown for 60-66 h in contact with COS cells transfected with either sense or antisense *shh* expression constructs. (-) indicates that fewer than 0.5%, (+) 5-35%, (++) 35-80%, (+++) >90% of cells expressed the marker, n.d., not determined. Results were obtained from over 30 explants in each case.

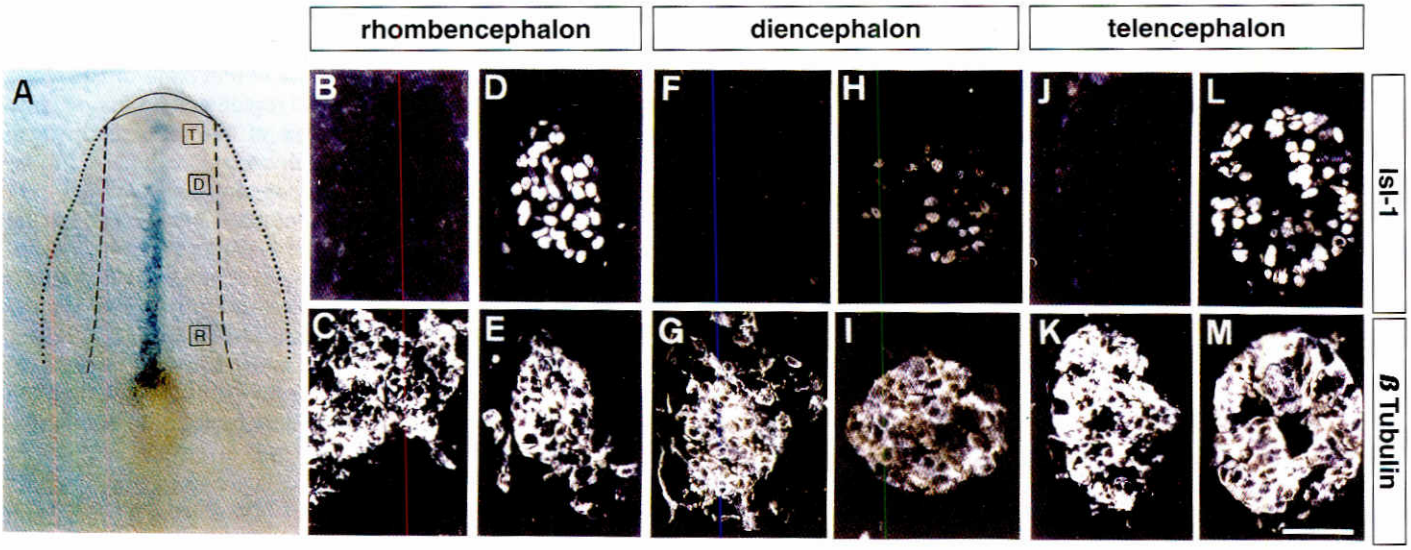

Fig. 3. shh induces Islet-1⁺ neurons in explants derived from different rostrocaudal levels of the neural plate. (A) *Expression of shh mRNA in the cells at the midline of a HH stage 6 chick embryo shown by whole-mount in situ hybridization. Sections through such embryos show that shh mRNA is expressed both in neural ectoderm and in the underlying mesoderm (data not shown). The position of the prospective telencephalic (T), diencephalic (D) and rhombencephalic (R) regions of the neural plate isolated for in vitro assays is indicated. The head-fold is at the top and the approximate neuroectodermal/ectodermal border is indicated by a dashed line. Dotted line indicates approximate border of the epiblast. Immunofluorescence micrographs in* **B-M** *show explants cultivated for 65 h on COS cells transfected with antisense or sense shh cDNA.* **(B,C)** *Section of a rhombencephalic level explant grown on COS cells transfected with antisense shh. No Islet-1⁺ cells are detected* **(B)** *even though ß-tubulin⁺ neurons have differentiated* **(C). (D,E)** *Section of a rhombencephalic level explant grown on COS cells transfected with sense shh. Numerous Islet-1⁺ cells are detected* **(D)** *virtually all of which coexpress ß-tubulin* **(E). (F,G)** *Section of a diencephalic level explant grown on COS cells transfected with antisense shh. No Islet-1⁺ cells are detected* **(F)** *even though ß-tubulin⁺ neurons are present* **(G). (H,I)** *Section of a diencephalic level explant grown on COS cells transfected with sense shh. Numerous Islet-1⁺ cells are present, and these coexpress ß-tubulin⁺* **(I). (J,K)** *Section through a telencephalic level explant grown on COS cells transfected with antisense shh. No Islet-1⁺ cells are detected* **(J)** *despite the differentiation of ß-tubulin⁺ neurons* **(K). (L,M)** *Section of a telencephalic level explant grown on COS cells transfected with sense shh. Numerous Islet-1⁺ cells are present* **(L)**, *and these coexpress ß-tubulin* **(M).** *Scale bar: A, 250 μm, B-M, 25 μm. Reproduced with permission from Cell.*

express Lim-1 (Table 1) and thus correspond phenotypically to neurons characteristic of the diencephalon (Fig. 2Aii). In contrast, in both rhombencephalic and telencephalic level explants, the Islet-1⁺ neurons induced by Shh do not express Lim-1 (Table 1).

Taken together, these results show that Shh induces ventral neuronal cell types in prospective forebrain regions of the neural plate and that these neurons express marker combinations appropriate for distinct classes of ventral neurons that are generated ventrally in both the diencephalon and telencephalon (Ericson *et al.*, 1995).

Discussion

A vertebrate homolog of the Drosophila *hedgehog* gene, *shh*, is expressed by the notochord and floor plate and can mimic the ability of these two midline cell groups to induce motor neuron differentiation (Roelink *et al.*, 1994). Shh has, therefore, been implicated in the induction of ventral neuronal types at caudal levels of the neuraxis. *shh* is also expressed by cells in the region of the diencephalon rostral to the floor plate and also in the ventral telencephalon (Echelard *et al.*, 1993; Krauss *et al.*, 1993; Chang *et al.*, 1994; Roelink *et al.*, 1994) and we have found that shh induces the differentiation of ventral neuronal cell types characteristic of the diencephalon and telencephalon in regions of the neural plate that normally give rise to these two subdivisions of the forebrain (Ericson *et al.*, 1995).

The LIM homeodomain protein Islet-1, an early marker of motor neuron differentiation at caudal levels of the neural tube, is also induced by shh early in the differentiation of these ventral diencephalic and telencephalic neurons. Islet-1⁺ neurons, however, have distinct regional identities that appear to be constrained by the axial level of origin of cells within the neural plate. Thus, a single inducing molecule, Shh, may participate in the differentiation and diversification of ventral neuronal cell types along the entire rostrocaudal extent of the neural tube acting on neural plate cells of predetermined rostrocaudal character (Ericson *et al.*, 1995).

Early restriction in the rostrocaudal character of neural plate cells

Embryological studies have provided evidence that the rostrocaudal and dorsoventral character of cells within the neural plate and neural tube is controlled by independent patterning systems (Doniach *et al.*, 1992; Jessell and Dodd, 1992; Ruiz i Altaba, 1992; Smith, 1993). The early rostrocaudal character of neural cells appears to be established prior to the definition of cell identity along the dorsoventral axis of the neural tube (Roach, 1945; Jacobson, 1964; Simon *et al.*, 1995). The results presented here support this idea and in addition show that the

rostrocaudal character of neural cells that has been defined at the neural plate stage is maintained *in vitro*, both in the absence and presence of ventralizing signals mediated by shh. Thus, an early and stable restriction in the potential of cells located at different rostrocaudal positions within the neural plate appears to define the repertoire of ventral neuronal cell types that can be generated upon exposure of cells to shh.

The signals that establish the early rostrocaudal character of neural plate cells have not been identified. However, studies in several vertebrate species have provided evidence that the action of these signals subdivides the neural tube along its rostrocaudal axis, into discrete domains or segments (Vaage, 1969; Lumsden and Keynes 1989; Figdor and Stern, 1993). Many or all of these segmental domains coincide with the boundaries of expression of transcription factors (MacDonald *et al.,* 1994; Rubenstein *et al.,* 1994). The intrinsic restriction in the potential fates of neural plate cells might, therefore, be established by the early and regionalized expression of transcription factors that later reveal segmental subdivisions of the neural tube.

Homeobox gene expression and a common program for the generation of ventral neurons

The detection of Islet-1 in ventral neuronal cell types generated at many different positions along the rostrocaudal extent of the neural tube suggests that the expression of this gene is more closely associated with the differentiation of neurons of ventral character than with the generation of any specific class of ventral neuron. However, at rhombencephalic and mesencephalic levels, the differentiation of serotonergic and dopaminergic neurons can be induced by the notochord and floor plate but these neurons do not express Islet-1 (Yamada *et al.,* 1991; Hynes *et al.,* 1995 and our unpublished observations). Thus, although Islet-1 expression is a prominent marker of ventral neuronal differentiation, its expression is not always associated with the generation of ventral neuronal cell types that depend on notochord- and floor plate-derived signals.

Nevertheless, the expression of Islet-1 by many distinct classes of ventral neurons raises the possibility that elements of the response of neural plate cells to shh may be conserved along the rostrocaudal axis. In support of this, members of the *Nkx 2* family of homeobox genes, notably *Nkx 2.1* and *Nkx 2.2* are expressed in the ventral neural tube at all rostrocaudal levels, in a domain that overlaps closely with that of *shh* (Lazzaro *et al.,* 1991; Price *et al.,* 1992; Rubenstein *et al.,* 1994). Moreover, at forebrain levels the expression of both Nkx 2.1 and Nkx 2.2 (Barth and Wilson, 1995) is induced by shh. Thus, the Nkx 2 and Islet-1 homeodomain proteins might represent elements of a common Shh-response program that is activated in neural plate cells independent of their rostrocaudal position.

Taken together, these studies implicate Shh in the induction of ventral neuronal cell types along the entire rostrocaudal extent of the embryonic central nervous system. Several prominent classes of neurons that are depleted in neurodegenerative diseases derive from ventrally-located progenitors at different axial levels of the neural tube: motor neurons at spinal levels, dopaminergic neurons at mesencephalic levels and striatal and basal forebrain neurons at telencephalic levels. Since shh appears to direct the ventral neuronal fates of progenitor cells during embryogenesis, the protein might exert a similar activity on neuronal progenitors present in the adult (Reynolds and Weiss, 1992) and thus could repopulate the central nervous system with classes of ventral neurons depleted in neurodegenerative disease.

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