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Functional expression of the tachykinin NK_1 receptor by floor plate cells in the embryonic rat spinal cord and brainstem

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1. The floor plate is a ventral mid-line structure that plays a pivotal role in the organization of the developing vertebrate central nervous system. Previous studies have demonstrated that the floor plate may provide signals that induce neuronal differentiation and guide axons; however, it is not known whether the floor plate can itself respond to signals that derive from surrounding tissue.

2. The peptide substance P is one of the first transmitters to be expressed in the developing spinal cord. To determine whether the floor plate may respond to substance P we have examined the expression of the principal substance P receptor (the tachykinin NK_1 receptor) by floor plate cells of the rat embryonic spinal cord using immunocytochemistry, *in situ* hybridization and fura-2 calcium imaging.

3. Immunocytochemistry demonstrated selective expression of the NK_1 receptor by cells at the ventral mid-line of the spinal cord. Double immunofluorescence labelling with the specific floor plate marker FP3 indicated that NK_1 receptor expression is confined to cells in the lateral region of the floor plate.

4. In order to confirm the specificity of the NK_1 receptor immunoreactivity we performed *in situ* hybridization histochemistry using antisense cRNA probes directed against the NK_1 receptor. *In situ* hybridization demonstrated selective expression of NK_1 receptor mRNA by floor plate cells.

5. The ontogeny of NK_1 receptor protein and mRNA expression in the floor plate was defined. NK_1 receptor expression occurred in a rostrocaudal progression that begins at embryonic day 10–11 (E10–E11) and is complete by E12–E14. The restriction of NK_1 receptor expression to the lateral part of the floor plate was conserved throughout embryonic development.

6. NK_1 receptor signalling was assessed by monitoring substance P-evoked changes in the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) of acutely dissociated cells from the floor plate region. Application of substance P (5 nM) elevated $[Ca^{2+}]_i$ in 10 % of cells examined.

7. Selective neurokinin agonists were used to identify the receptor subtype involved in the substance P-evoked elevation of $[Ca^{2+}]_i$. Acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P(6–11) (5 nM) and [Sar^9,$Met(O_2)^{11}$]-substance P (5 nM), two highly selective NK_1 receptor agonists, both elevated $[Ca^{2+}]_i$ in floor plate cells that responded to substance P. [β-Ala^8]-neurokinin A(4–10) (50 nM) and senktide (50 nM), selective agonists respectively of NK_2 and NK_3 receptors, had no effect on $[Ca^{2+}]_i$.

The peptide substance P is a widely distributed tachykinin that participates in an array of physiological processes. In the mature spinal cord substance P is released from primary afferent terminals in the dorsal horn and has been implicated in the transmission of nociceptive sensory information (Willis & Coggeshall, 1991). During the development of the spinal cord, substance P is expressed transiently by several distinct classes of cells, raising the possibility that it might have a role in spinal cord development (Senba *et al.* 1982; Marti *et al.* 1987; Ni &

Jonakait, 1988). Moreover *in vitro* studies have shown that substance P can enhance neurite outgrowth and promote cell migration and proliferation (Narumi & Fujita, 1977; Narumi & Maki, 1978). However, the physiological role of substance P during the development of the nervous system, if any, remains unknown.

The time course of substance P expression in the developing spinal cord overlaps with the presence of the floor plate, a specialized group of cells at the ventral midline of the spinal cord, hindbrain and midbrain (Kingsbury, 1930). Previous studies have provided evidence that the floor plate serves as a source of signals that control neural cell fate within the embryonic spinal cord (Placzek, Yamada, Tessier-Lavigne, Jessell & Dodd, 1991; Yamada, Placzek, Tanaka, Dodd & Jessell, 1991; Jessell & Dodd, 1992; Placzek, Jessell & Dodd, 1993; Yamada, Pfaff, Edlund & Jessell, 1993; Roelink *et al.* 1994). The floor plate also provides signals that regulate the directed growth and surface properties of commissural axons (Tessier-Lavigne, Placzek, Lumsden, Dodd & Jessell, 1988; Bovolenta & Dodd, 1990, 1991; Placzek, Tessier-Lavigne, Jessell & Dodd, 1992; Hatta, 1992; Dodd & Jessell, 1993). However, it is not known whether the floor plate, in addition to providing signals, can itself respond to signals that derive from surrounding tissues. Because substance P expression (Senba *et al.* 1982; Marti *et al.* 1987; Ni & Jonakait, 1988) overlaps with the developmental activities of the floor plate, it is possible that substance P might influence floor plate properties. Until recently, however, the identification of the embryonic targets of substance P has been hindered by the lack of probes that can localize substance P receptors with precision.

The physiological actions of substance P are mediated primarily by interactions with the NK_1 receptor. Two additional receptors, the NK_2 and NK_3 receptors, primarily mediate the actions of the tachykinins neurokinin A and neurokinin B. The cloning and sequencing of these tachykinin receptors (Krause, Blount & Sachais, 1994) has permitted the development of molecular probes that can define the distribution of NK_1 receptor with precision. To examine further the potential role of substance P during the development of the spinal cord we therefore used RNA probes and antibodies to define the distribution of NK_1 receptor mRNA and protein. In order to determine whether the immunocytochemically detected NK_1 receptor is in fact functional, we also used fura-2 calcium imaging to monitor elevations of $[Ca^{2+}]_i$ evoked by substance P.

Part of this study has been published in abstract form (Heath, Lee & Dodd, 1994).

METHODS

All embryos were obtained from timed-mated Sprague–Dawley rats (viral antibody free; Hilltop Laboratories, Scottsdale, PA, USA) that were killed by CO_2 asphyxiation. Gravid uteri were rapidly removed and the embryos were delivered into L-15 medium at 4 °C and decapitated. Embryos used for immunocytochemistry and *in situ* hybridization were fixed whole (E9, 10, 11, 12, 14) or after evisceration (E16, 17, 19, 20). Embryos used for preparing acutely dissociated floor plate cells were killed by decapitation prior to dissection.

Immunocytochemistry

Embryos were fixed in ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7·4. The tissue was cryoprotected with 30% sucrose in PBS, mounted in Tissue-tek (Miles Inc., Elkhart, IN, USA), and cut into 12–15 μm sections on a cryostat. Sections from all levels of the central nervous system were incubated overnight in primary antiserum at dilutions of 1:1000 to 1:10000 carried out in PBS with 0·3% Triton X-100 and 1% heat-inactivated goat serum at 4 °C. Antiserum directed against the NK_1 receptor has been characterized (Vigna *et al.* 1994) and was a generous gift from Dr S. Vigna (Duke University, Durham, NC, USA). Monoclonal antibody FP3 (Placzek *et al.* 1993) was diluted 1:1 with PBS. Some sections were processed with biotinylated secondary antibody, avidin–biotin–peroxidase, and diaminobenzidine tetrahydrochloride chromogen (Vector Laboratories, Burlingame, CA, USA). Other sections were processed with fluorescein isothiocyanate (FITC)- and rhodamine isothiocyanate (RITC)-conjugated secondary antibodies (TAGO Inc., Burlingame, CA, USA).

In situ hybridization

Whole-mount *in situ* hybridization analysis of the rat embryonic tissue was performed essentially as previously described (Roelink *et al.* 1994). Sense and antisense digoxigenin–UTP-labelled cRNA probes corresponding to nucleotides 637–1224 of the NK_1 receptor (Hershey, Dykema & Krause, 1991) were synthesized from the plasmid pBS-SPR/si7 (a generous gift of J. Krause, Washington University, School of Medicine, St Louis, MO, USA). Antisense cRNA probes to the NK_3 receptor were synthesized from the plasmid pBS.rNKBR (McCarson & Krause 1994) containing the 3′ coding region of the NK_3 receptor (nucleotides 1014–1359) (Shigemoto, Yokata, Tsuchida & Nakanishi, 1990). Embryos were embedded in agarose and 50 μm sections were cut using an Oxford Vibratome. Specific hybridization was not detected when the sense NK_1 receptor probe or the NK_3 receptor antisense probe was used.

Measurement of $[Ca^{2+}]_i$

E16 floor plates were dissected in L-15 medium (Gibco) and incubated in S-MEM (minimal essential medium modified for suspension culture) with 0·064% trypsin (type III; Sigma, St Louis, MO, USA) and 80 mg ml^{-1} DNase (type IV, from bovine pancreas; Sigma) for 20 min at 37 °C. The floor plate is readily distinguished as a lucent band lying between the more opaque tissue of the ventral plate; however, it is likely that a small amount of tissue immediately lateral to the floor plate was inadvertently included in the dissection. The floor plates were washed with L-15 medium and dissociated mechanically in the presence of DNase and allowed to adhere to coverslips coated with poly-D-lysine.

After 20 min, cells were loaded with 5 μM fura-2 AM (Molecular Probes, Eugene, OR, USA) in 0·02% pluronic acid for 15 min at 22 °C. The perfusion bath contained (mM): NaCl, 145; KCl, 5; $MgCl_2$, 2; $CaCl_2$, 2; Hepes, 10; glucose, 0·1%; sucrose to 320 mosmol l^{-1}; pH 7·30. Substance P, acetyl-[Arg^6,Sar^9,Met(O$_2$)^{11}]-substance P(6–11), [Sar^9,Met(O$_2$)^{11}]-substance P, [β-Ala^8]-neurokinin A and senktide (Regoli *et al.* 1994) (all from Sigma) were applied in the perfusion bath for 10 s. Agonists were applied at intervals of 10 min; preliminary experiments suggested that responses desensitized for 1–4 min after agonist application. Agonists were rapidly washed out with a laminar flow of approximately 500 μl s^{-1} through a chamber volume of approximately 400 μl.

Fura-2 was excited by 75 W xenon bulb emission passed through 350 and 380 nm filters mounted in a Sutter filter wheel (Novato, CA, USA). Emission fluorescence was intensified by a Hamamatsu image intensifier and collected by a Hamamatsu CCD camera. Images were digitized and processed by a Videoprobe system (ETM Systems, Irvine, CA, USA) which also controlled the filter wheel. Background values were collected at each wavelength from a region between cells or from a cell-free field. $[Ca^{2+}]_i$ was computed on-line using the equation $[Ca^{2+}]_i = K_d S_f (R - R_{min})/(R_{max} - R)$, in which K_d is the apparent dissociation constant of fura-2 for Ca^{2+}, S_f is the scale factor of the optical system, R is the measured ratio of fluorescence at 350 and 380 nm, R_{min} is the fluorescence ratio in the presence of zero Ca^{2+} and R_{max} is the fluorescence ratio in the presence of a saturating (1 mM) concentration of Ca^{2+}. A series of EGTA-buffered solutions containing standardized concentrations of Ca^{2+} and 100 μM fura-2 pentapotassium salt was used to calibrate this system. The solutions were placed in 20 μm precision path length rectangular capillary tubes and background-subtracted ratiometric measurements obtained. This *in vitro* calibration protocol gave an apparent K_d of 255 nM, which is in agreement with previously described values (Grynkiewicz, Poenie & Tsien, 1985). Mean values of $[Ca^{2+}]_i$ elevation are expressed ± standard deviation.

RESULTS

NK$_1$ receptor is expressed by a subset of cells in the floor plate

Immunocytochemistry was performed on rat embryos to identify sites of NK$_1$ receptor expression. The NK$_1$ receptor was expressed selectively in a restricted area of the ventral spinal cord that appeared to correspond to the floor plate (Fig. 1). To determine whether cells that express NK$_1$ receptor were contained entirely within the floor plate, double-immunofluorescence labelling was performed with the monoclonal antibody FP3, which recognizes a surface antigen selectively expressed on floor plate cells over the full mediolateral extent of the floor plate (Placzek, Jessell &

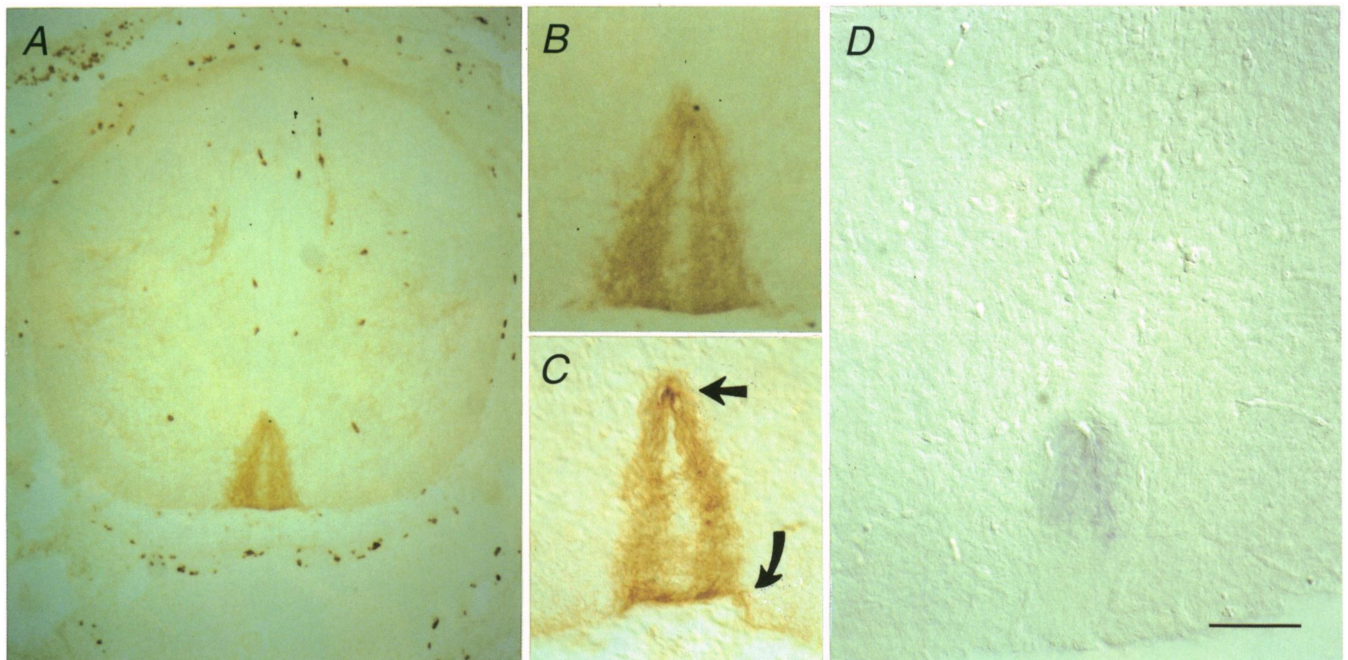

Figure 1. NK$_1$ receptor protein and mRNA expression in E16 thoracic spinal cord

A, immunocytochemical demonstration of NK$_1$ receptor expression in the region of the floor plate. *B*, higher magnification photograph of floor plate shown in *A*. *C*, NK$_1$ receptor expression in the floor plate region illustrating the lack of antigen in the medial part of the floor plate. Intense staining of the apical region, which forms the ventral wall of the central canal, and of the subpial region is apparent. *D*, *in situ* hybridization of NK$_1$ receptor transcripts in the apical region of the floor plate. Scale bar: *A*, 300 μm, *B–D*, 150 μm.

Dodd, 1993). Cells and processes that expressed the NK_1 receptor were restricted to the floor plate, as defined by FP3 immunoreactivity (Fig. 2). With the exception of the ventral surface of the spinal cord, however, the expression of NK_1 receptor was confined to the lateral region of the floor plate; the medial region of the floor plate exhibited no detectable antigen. This result provides evidence that the floor plate does not constitute a homogeneous population of cells. Fine processes expressing NK_1 receptor radiate from the apical region near the central canal towards the ventral surface of the spinal cord where they cross the mid-line. This pattern was conserved throughout the rostrocaudal extent of the floor plate.

Messenger RNA encoding NK_1 receptor is present in the floor plate

To confirm that the immunoreactivity we detected reflected accurately the expression of NK_1 rather than a cross-reacting antigen, we performed *in situ* hybridization using an antisense cRNA probe directed against NK_1 receptor. NK_1 receptor mRNA was present in the apical region of the floor plate near the central canal (Figs 1, 3 and 4). Hybridization was not detected with NK_1 receptor sense probe or with a probe to NK_3 receptor. The correspondence between the labelling patterns generated by immunocytochemistry and by *in situ* hybridization

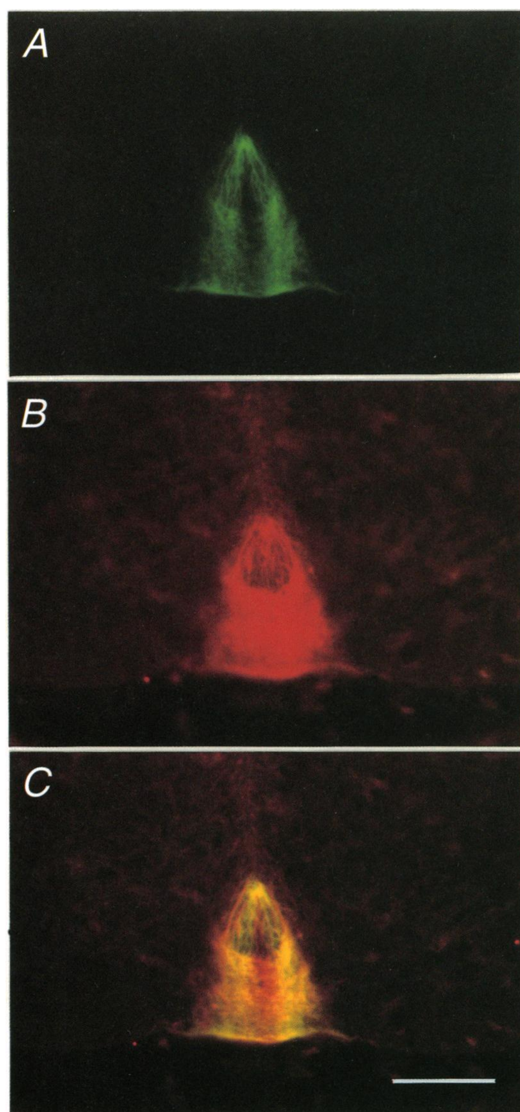

Figure 2. NK_1 receptor expression is confined to the floor plate

A, NK_1 receptor expression in the E16 thoracic spinal cord. *B*, delineation of the full mediolateral extent of the floor plate by labelling with monoclonal antibody FP3. *C*, double-labelling demonstrates that the pattern of NK_1 receptor expression does not extend beyond the lateral limit of FP3 expression. Scale bar: *A–C*, 200 μm.

confirms that NK$_1$ receptor mRNA and protein are expressed by the floor plate. In older embryos the expression of mRNA was confined to a smaller region than the expression of receptor protein, suggesting that the receptor is exported to distal cellular sites after translation occurs. This was particularly evident at the level of the brainstem at E14 (Fig. 4). Floor plate cells located near the central canal extend lamellar processes through the full extent of the floor plate to the subpial surface of the spinal cord (Yoshioka & Tanaka, 1989), providing a probable anatomical substrate for the relationship observed here.

Ontogeny of NK$_1$ receptor and NK$_1$ receptor mRNA expression in the floor plate

Next we determined the time course of NK$_1$ receptor expression. As is seen with other floor plate markers, the time course of NK$_1$ receptor displayed a rostrocaudal progression. Faint NK$_1$ receptor immunoreactivity appeared in the brainstem and rostral levels of the spinal cord at E10–E11 (Fig. 3) and extended caudally throughout the length of the spinal cord by E12–E14. The level of NK$_1$ receptor expression in the spinal cord reached peak intensity by E14–E16, and the derivatives of floor plate cells that form the ventral raphe of the spinal cord express

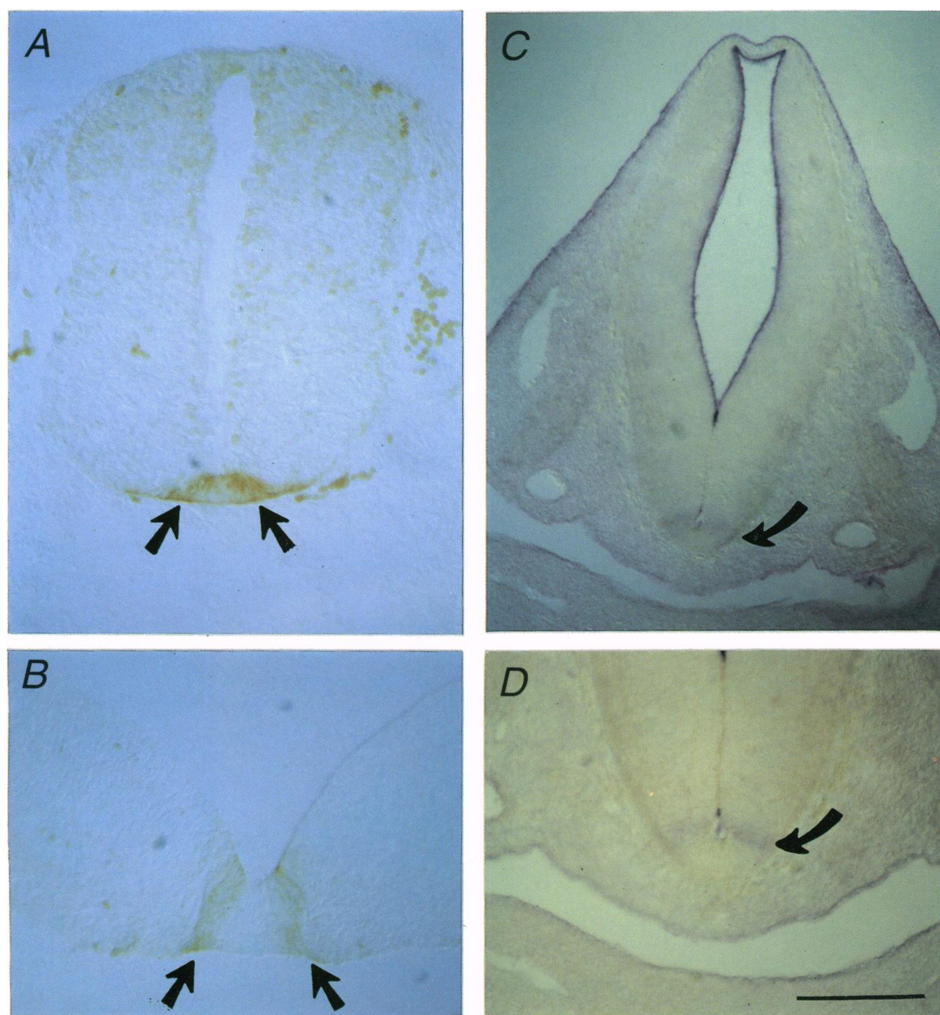

Figure 3. NK$_1$ receptor protein and mRNA expression in E11–E12 floor plate

The straight arrows indicate the lateral region of the floor plate where NK$_1$ receptor protein is expressed (*A* and *B*). The curved arrows indicate the region of NK$_1$ receptor transcript expression (*C* and *D*). *A*, NK$_1$ receptor protein expression in the floor plate of the cervical spinal cord at E11. *B*, NK$_1$ receptor protein expression in the floor plate of the hindbrain at E11. *C*, NK$_1$ receptor transcript in the E12 brainstem. *D*, higher magnification photograph of ventral brainstem seen in *C*. Scale bar: *A*, *B* and *D*, 100 μm; *C*, 200 μm.

trace NK_1 receptor until post-embryonic day 7–10 (P7–P10). The restriction of NK_1 receptor to lateral floor plate cells persisted throughout embryonic development.

Hybridization to NK_1 receptor mRNA was first seen in the brainstem and cervical spinal cord at E12 (Fig. 3) and became more intense by E14–E16, following a rostrocaudal course of expression similar to that seen using immunocytochemistry. The advent of detectable hybridization lagged behind the NK_1 receptor immunoreactivity; this, presumably, represents a sensitivity difference between the probes. At early stages (E12) the mRNA expression extended from the apical to the basal surface of the floor plate (Fig. 3), while at later stages it was confined to the apical region close to the central canal.

Substance P activates floor plate NK_1 receptors and promotes elevation of $[Ca^{2+}]_i$

Although the immunocytochemical and *in situ* hybridization studies described above document the presence of NK_1 receptors in the floor plate, they do not address whether these receptors are functional. Because tachykinin receptors are coupled to intracellular calcium signalling in a wide variety of cell types (Putney, 1994), we used fura-2 calcium imaging to determine whether NK_1 receptors on floor plate cells are functional. To avoid artifacts generated by the phenotypic instability of some neural cell types *in vitro*, we studied acutely isolated floor plate cells within 4 h of dissociation.

Elevation of $[Ca^{2+}]_i$ in response to a 10 s application of 5 nM substance P occurred in 10% of cells tested (31 of 312). An example of a substance P-responsive floor plate cell is shown in Fig. 5. The low proportion of responsive cells in the preparation could reflect the proportion of floor plate cells expressing functional NK_1 receptors, the selective death of these cells due to the tissue dissociation protocol, or the inclusion in the dissection of cells outside the floor plate. The mean resting $[Ca^{2+}]_i$ was 84 ± 35 nM, and the mean rise in $[Ca^{2+}]_i$ in response to 5 nM substance P was 545 ± 474 nM.

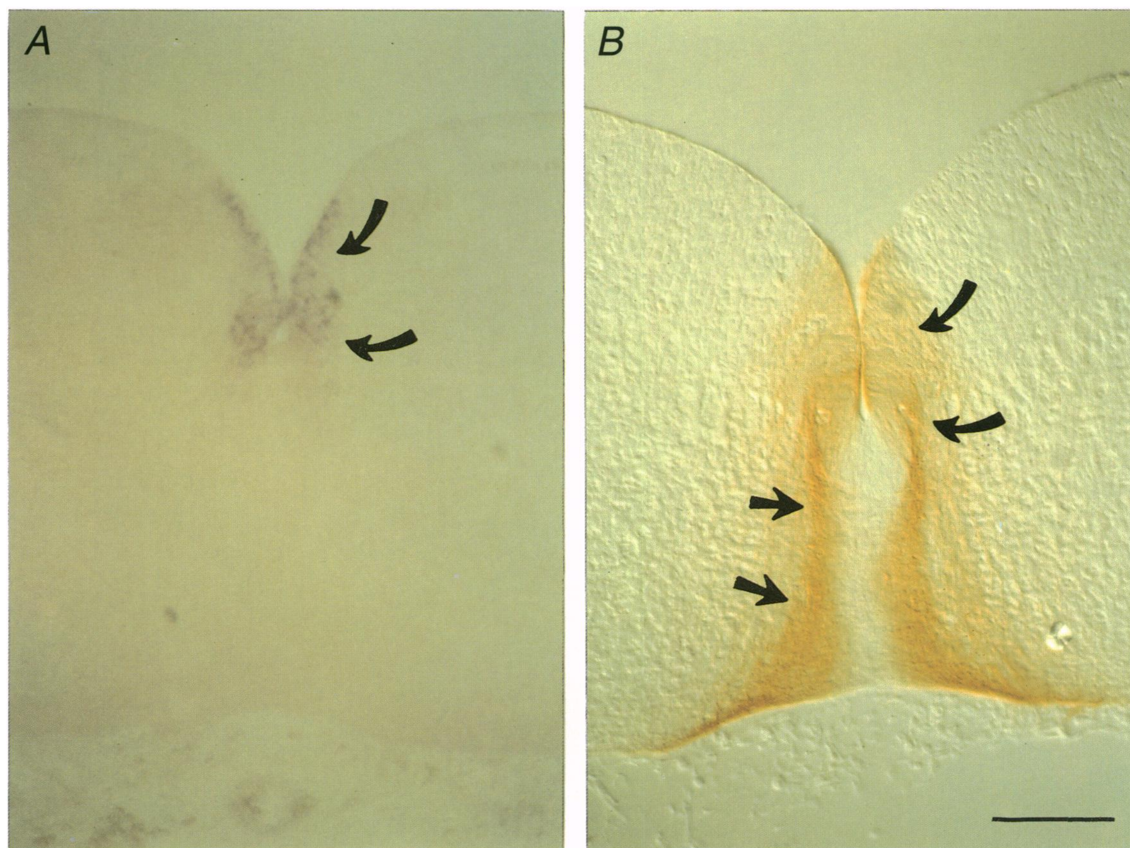

Figure 4. E14 brainstem. NK_1 receptor protein expression extends ventrally beyond the region of NK_1 mRNA expression

A, NK_1 mRNA expression, indicated by the curved arrows, is confined to floor plate cells immediately underlying the fourth ventricle (which corresponds to the central canal of the spinal cord). *B*, immunocytochemical localization of NK_1 receptor protein in a corresponding section from another animal. The curved arrows indicate the region occupied by floor plate cell bodies, and the straight arrows indicate the region of floor plate cell processes. Scale bar: 150 μm.

Although substance P interacts principally with the NK_1 receptor, at high concentration substance P is also capable of activating NK_2 and NK_3 receptors. To classify more precisely the receptor type involved in substance P-evoked elevation of $[Ca^{2+}]_i$ in floor plate cells, selective agonists of NK_1, NK_2 and NK_3 receptors were used. Two substance P analogues that are highly selective agonists for the NK_1 receptor, acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P (6–11) and [Sar^9,$Met(O_2)^{11}$]-substance P (Regoli *et al.* 1994), were tested on six cells that responded to substance P. Each of these cells also responded to both of the NK_1 selective agonists (5 nM). These two substance P analogues are inactive at NK_2 and NK_3 receptors (Regoli *et al.* 1994), indicating that substance P activates NK_1 receptors on floor plate cells. The mean rises in $[Ca^{2+}]_i$ in response to 5 nM acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P(6–11) and to 5 nM [Sar^9,$Met(O_2)^{11}$]-substance P were 893 ± 733 and 889 ± 708 nM, respectively.

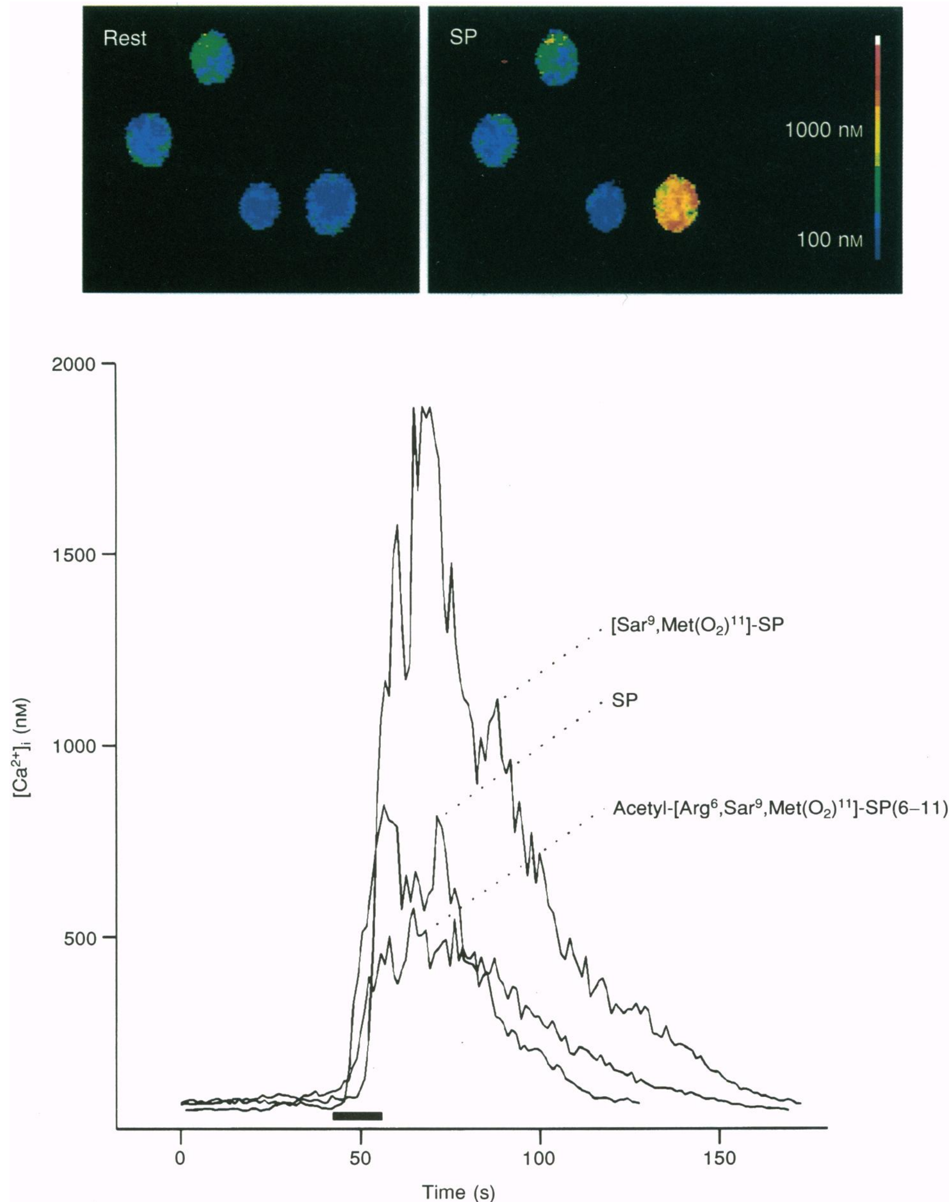

Figure 5. Elevation of $[Ca^{2+}]_i$ in floor plate cells by substance P and NK_1 selective agonists

Acutely dissociated floor plate cells were monitored by fura-2 imaging (colour insets). Responses to agonists are shown for the cell in the lower right of the insets; the surrounding cells did not respond. The bar indicates the duration of drug application. Cells were exposed sequentially at intervals of 10 min to substance P (SP; 5 nM), acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P(6–11) (5 nM) and [Sar^9,$Met(O_2)^{11}$]-substance P (5 nM).

Further confirmation of the role of NK_1 receptors in mediating the substance P-evoked elevation of $[Ca^{2+}]_i$ in floor plate cells has been derived from the use of the selective NK_2 agonist [β-Ala^8]-neurokinin A and the selective NK_3 agonist senktide (Regoli *et al.* 1994). Four cells that responded to the selective NK_1 agonist [Sar^9,Met(O_2)^{11}]-substance P (5 nM) exhibited no response to 50 nM [β-Ala^8]-neurokinin A(4–10) and 50 nM senktide (Fig. 6). The NK_2 and NK_3 selective agonists were applied at concentrations designed to elicit maximal activation of their preferred receptors without activating NK_1 receptors (Regoli *et al.* 1994). This result indicates that of the tachykinin receptors that are known to be activated by substance P, in the floor plate only the NK_1 receptor contributes to the elevation of $[Ca^{2+}]_i$ evoked by substance P.

DISCUSSION

These studies demonstrate that the NK_1 tachykinin receptor is expressed in a subset of floor plate cells in the embryonic rat spinal cord. The specificity of the antiserum used to localize NK_1 receptor was confirmed by the congruence in the patterns of NK_1 receptor mRNA and protein expression. Substance P caused an elevation of $[Ca^{2+}]_i$ in cells dissociated from the floor plate region, indicating that the NK_1 receptor is functional and is coupled to intracellular signalling mechanisms. Two selective NK_1 receptor ligands also elevated $[Ca^{2+}]_i$ in floor plate cells, whereas selective agonists of NK_2 and NK_3 receptors were inactive, suggesting that the effects of substance P on floor plate cells are mediated solely by NK_1 receptors.

The pattern of substance P expression in the developing spinal cord has been described (Senba *et al.* 1982; Marti *et al.* 1987; Ni & Jonakait, 1988) and several distinct neural cell types may serve as sources of peptide that could activate NK_1 receptors on floor plate cells. Substance P-containing cells first appear immediately lateral to the floor plate and extend fibres through the floor plate and across the mid-line (Ni & Jonakait, 1988). Neural cells containing substance P also appear in the ventral horn, in the ventral aspect of the dorsal horn, and in the developing ventral white matter (Senba *et al.* 1982).

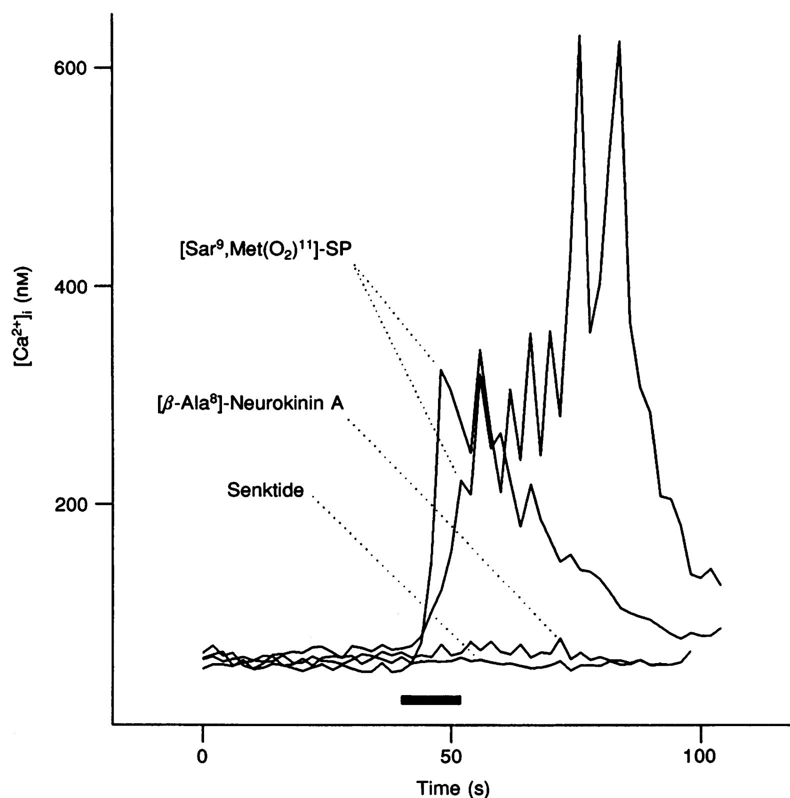

Figure 6. Elevation of $[Ca^{2+}]_i$ in floor plate cells by an NK_1 selective agonist but not by NK_2 and NK_3 selective agonists

A floor plate cell was exposed at 10 min intervals to selective neurokinin receptor agonists. The bar indicates the duration of drug application. The NK_1 selective agonist [Sar^9,Met(O_2)^{11}]-substance P (5 nM) elevated $[Ca^{2+}]_i$ whereas the selective NK_2 agonist [β-Ala^8]-neurokinin A (50 nM) and the selective NK_3 agonist senktide (50 nM) failed to elicit a response. The NK_2 and NK_3 selective agonists were applied between the two [Sar^9,Met(O_2)^{11}]-substance P applications; the larger response to [Sar^9,Met(O_2)^{11}]-substance P occurred the second time it was applied.

The possibility that substance P might play a role in spinal cord development has also been raised by previous studies of substance P binding and physiology. While binding assays have provided evidence for substance P receptors in the embryonic central nervous system (Quirion & Dam, 1986), there are no autoradiographic data to define their location with respect to the floor plate. The distribution of substance P binding sites undergoes major modifications during the postnatal period, but this occurs after the time when the floor plate is known to provide developmental cues (Charlton & Helke, 1986). Studies of dissociated embryonic and neonatal spinal cords have shown that immature glial cells express substance P receptors (Cholewinski, Hanley & Wilkin, 1988; Heath, Womack & MacDermott, 1994), but again provide no anatomical information about sites of substance P receptor expression. Because the floor plate displays well-characterized developmental activities, the recognition that it expresses functional NK_1 receptors now provides a basis for the further study of the role of substance P in embryonic development.

The embryonic spinal cord also expresses a wide variety of peptide and amine transmitters, including Leu-enkephalin, neurotensin, somatostatin, calcitonin gene-related peptide, galanin, vasoactive intestinal polypetide and serotonin (Senba et al. 1982; Marti et al. 1987; Ni & Jonakait, 1988). However, it is not known whether the floor plate expresses receptors for any of these modulators.

Three lines of evidence provide a basis for classifying the floor plate into distinct medial and lateral domains. Lipocortin 1 (also called p35) is a marker that delineates a boundary between the medial and lateral floor plate (McKanna & Cohen, 1989). Lipocortin 1 is present only in the medial one-third of the floor plate, and thus probably defines a set of floor plate cells complementary to those expressing the NK_1 receptor. A mediolateral distinction in floor plate cells has also been noted in studies on the expression of a mouse lacZ transgene that is expressed selectively by lateral floor plate cells (Campbell & Peterson, 1993). Although the lateral cells of the floor plate are the last to complete cell division (Altman & Bayer, 1984), it has not been possible to assign distinct functions to subsets of floor plate cells. The identification of NK_1 receptors on these cells raises the possibility that medial and lateral floor plate cells, because of their differing sensitivities to substance P, may serve different developmental functions.

Because of the importance of the floor plate in organizing cell identity and axonal trajectories in the spinal cord, the presence of functional NK_1 receptor on floor plate cells suggests that substance P might have a role in spinal cord development. The physiological demonstration of functional NK_1 receptors in the floor plate raises the possibility that, in addition to its function as a source of signals that co-ordinate the development of the spinal cord, the floor plate may respond to signals that derive from surrounding tissues. *In vitro* experiments suggest that the ability of the floor plate to specify the phenotype of surrounding cells and regulate axon guidance does not require maintained external signals (Placzek et al. 1991). Nevertheless, it remains possible that the levels of expression of inductive and axonal guidance molecules provided by the floor plate are regulated by extrinsic signals. Modulation of these activities by substance P and other peptide mediators could contribute to the fine tuning of developmental processes in the embryonic central nervous system.

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