Identification of an N-cadherin Motif That Can Interact with the Fibroblast Growth Factor Receptor and Is Required for Axonal Growth*

In this study, we show that the neurite outgrowth response stimulated by N-cadherin is inhibited by a recently developed and highly specific fibroblast growth factor receptor (FGFR) antagonist. To test whether the N-cadherin response also requires FGF function, we developed peptide mimetics of the receptor binding sites on FGFs. Most mimetics inhibit the neurite outgrowth response stimulated by FGF in the absence of any effect on the N-cadherin response. The exceptions to this result were two mimetics of a short FGFI1 sequence, which has been shown to interact with the region of the FGFR containing the histidine-alanine-valine motif. These peptides inhibited FGF and N-cadherin responses with similar efficacy. The histidine-alanine-valine region of the FGFR has previously been implicated in the N-cadherin response, and a candidate interaction site has been identified in extracellular domain 4 of N-cadherin. We now show that antibodies directed to this site on N-cadherin inhibit the neurite outgrowth response stimulated by N-cadherin, and peptide mimetics of the site inhibit N-cadherin and FGF responses. Thus, we can conclude that N-cadherin contains a novel motility motif in extracellular domain 4, and that peptide mimetics of this motif can interact with the FGFR.

N-cadherin is a member of the classical cadherin family of transmembrane glycoproteins that mediate cell-to-cell adhesion via a homophilic binding mechanism (1). Similar to other members of this family, the extracellular portion of the molecule is composed of five cadherin domains with a large body of evidence, suggesting that the homophilic binding site resides in the first extracellular domain (ECD1) (2, 3). In the nervous system, N-cadherin function has been implicated in a number of key events that range from the control of axonal growth and guidance (4, 5) to synapse formation and synaptic plasticity (6–9).

In addition to homophilic binding, cadherins have been shown to interact with a number of adaptor or signaling molecules. For example, the interaction of the cytoplasmic domain of the classical cadherins with the actin-based cytoskeleton, which is important for adhesion, is mediated by the catenins (10, 11). An interaction with a cell surface N-acetylglactosaminyolphosphotransferase has also been reported and implicated in N-cadherin function (12). More recently, the non-receptor protein tyrosine phosphatase PTP1B and the receptor protein tyrosine phosphatase PTPα have been shown to bind directly to the cytoplasmic domain of N-cadherin and other cadherins and modulate function by regulating tyrosine phosphorylation of the cadherin and/or catenins (13, 14). The notion, that the activation of signaling cascades in cells rather than adhesion per se might drive some N-cadherin responses, is supported by the recent observation that a soluble form of N-cadherin can promote axonal growth (15).

A number of groups have implicated the fibroblast growth factor receptor (FGFR) in N-cadherin function. For example, neurite outgrowth stimulated by N-cadherin has been shown to be inhibited by a wide variety of agents that inhibit FGFR function in neurons (16), including the expression of a dominant negative FGFR (15, 17, 18). In addition, N-cadherin can promote "contact-dependent" survival of ovarian granulosa cells in an FGFR-dependent manner (19). More recently, N-cadherin has been reported to promote the motility of cancer cells with some data suggesting that the FGFR might be involved in this response (20, 21). However, it remains unclear as to whether N-cadherin homophilic binding modulates FGF interactions with the FGFR (20), or whether the FGFR requirement reflects a more direct interaction among these molecules (22). In support of the latter possibility, it has recently been shown that the antibody clustering of N-cadherin in cells is associated with the co-clustering of the FGFR (15), and that the FGFR and N-cadherin will co-precipitate from cells (23, 24).

We have used the structures of a number of FGF/FGFR crystals to develop peptide mimetics of the receptor binding sites on FGFs. The majority of these peptides inhibit the neurite outgrowth response stimulated by FGF in the absence of any effect on the same response stimulated by N-cadherin. The exceptions to this finding are two mimetics of a short sequence on FGFI1 that interacts with the histidine-alanine-valine (HAV) region of the FGFRs; these peptides inhibited the FGF and N-cadherin responses with similar efficacy. Previous studies have implicated the HAV region of the FGFR in the N-cadherin response (16) and identified a candidate interaction motif in ECD4 of N-cadherin (22). In this study, we show that antibodies that bind to this motif in neurons specifically inhibit N-cadherin function. Furthermore, peptide mimetics of the N-cadherin motif inhibit N-cadherin and FGF function with sim-
A Novel Functional Motif in N-cadherin ECD4

**Peptide Synthesis and Purity and PD 173074—Synthetic peptides were obtained from commercial suppliers (AbCam Ltd, Mimotopes, or Multiple Peptide Systems). All peptides were purified by reverse-phase high pressure liquid chromatography and obtained at the highest level of purity (generally >95%). Where peptide sequences are underlined, the cysteine residues have been cyclized via a disulphide bond among the given cysteine residues. PD 173074 (31, 32) was prepared according to the general procedures described by Hamby et al. (33).**

**RESULTS**

The N-cadherin Response Is Dependent on the Catalytic Activity of the FGFR—A large body of evidence supports the view that neurite outgrowth stimulated by N-cadherin requires the presence of a functional FGFR in neurons (34). However, the validity of conclusions based on the use of kinase-deleted “dominant negative” forms of the FGFR have recently been challenged (35). To test whether the catalytic activity of the FGFR is required for the N-cadherin response, we took advantage of the recent development of a highly specific antagonist (PD 173074) that binds to the ATP binding pocket of the FGFR (31). We cultured rat cerebellar granule cells for ~18 h over monolayers of control 3T3 cells and monolayers of transfected 3T3 cells that express physiological levels of N-cadherin (the LK8 cell line). As expected, the neurons extended longer neurites on the N-cadherin-expressing monolayers (Fig. 1). This response was fully inhibited in a dose-dependent manner by PD 173074 (Fig. 1A). When cerebellar granule cells are grown over monolayers of 3T3 fibroblasts that express physiological levels of two additional cell adhesion molecules (NCAM and L1) or in the presence of PFG2, a neurite outgrowth response that is similar to the N-cadherin response is found (16, 36). PD 173074 also inhibited the neurite outgrowth response stimulated by NCAM, L1, and FGFR2 with a dose-response curve that is similar to the inhibition of the N-cadherin response (Fig. 1B). Arachidonic acid is a key second messenger in the signal transduction cascade that couples the activated FGFR to the neurite outgrowth response (37). When tested at up to 1 μM, PD 173074 does not inhibit the neurite outgrowth response stimulated by arachidonic acid (32), and this demonstrates that it has no nonspecific effects on neurite outgrowth at any downstream step in the pathway that couples the activated FGFR to the response.

The N-cadherin Response Does Not Require FGF Function—The fact that the FGFR is required for the N-cadherin response begs the question as to whether FGFs are also required for this response. Recent observations from a number of FGF/FGFR crystal complexes has led to a consensus on how FGFs interact with the receptor (27–30). The contact profile for the FGF1/FGFR2 interaction is shown in Fig. 2 with an essentially identical profile found for the other FGF/FGFR interactions (29). Four major linear clusters of amino acids on FGF1 account for most of the binding to the FGFR. It follows that peptide mimetics of these clusters might be capable of inhibiting FGF function by binding back to the receptors. In this study, we made linear peptide mimetics to three partially overlapping sites from cluster 1 and single peptide mimetics for clusters 2, 3, and 4. In addition, we made cyclic versions of two of the cluster 1 peptides. All of the peptides were initially tested at 100 μM in the neurite outgrowth response stimulated by FGF2 with the results summarized in Table I. Although all of the peptides showed significant inhibitory activity, peptide mimetics of cluster 1 and cluster 2 were the most active in the assay, and therefore, full dose response curves to these peptides were obtained (Fig. 3). The results show that these peptides can inhibit the FGF2 response with IC50 values ranging from ~20 to 65 μM. The data also clearly show that the peptides do not inhibit basal neurite outgrowth over 3T3 cell monolayers, and we can, therefore, conclude that
they do not have any nonspecific effects on neurons. Arachidonic acid is a key second messenger in the pathway that couples the activation of the FGFR to the neurite outgrowth response (36), and at the highest concentration tested (100 μg/ml), none of the above peptides inhibited the neurite outgrowth response stimulated by 10 μM arachidonic acid (data not shown). Thus, we can conclude that the above peptides inhibit FGFR responses at the level of ligand interaction with the FGFR.

In contrast to their effects on the neurite outgrowth response stimulated by FGFR2, 6 out of the 8 peptides had no effect on the N-cadherin response (Table I). Of the eight peptide mimetics of the FGFR binding sites, two inhibited the N-cadherin response just as well as the FGFR2 response (Table I and Fig. 4A). We analyzed the FGFR/FGFR crystals to identify potential binding sites for the peptides. In this context, both peptides (N-Ac-YCSNGGHF-NH2 and N-Ac-YCSNGGHFC-NH2) are mimetics of a short FGFR1 sequence that makes extensive and relatively exclusive contacts with the HAV region of the FGFRs (see Fig. 4B for details). In contrast, the other peptides are mimetics of sequences that make extensive contacts with the linker region between the Ig domain 2 and 3 or with Ig domain 3 (data not shown). Thus, we can conclude that although FGFR-derived peptides that target the HAV region of the FGFR can inhibit both FGFR and N-cadherin responses, a number of other peptides that target other regions of the FGFR exclusively inhibit the FGFR response.

Identification of a Novel Functional Motif in ECD4 of N-cadherin—Based on homology with peptide sequences that interact with the HAV region of N-cadherin, we have identified the IDPVNGQ motif from ECD4 of N-cadherin as a candidate binding motif for the HAV region of the FGFR (22). If the IDPVNGQ motif within ECD4 of N-cadherin is a functional motif capable of interacting with the HAV region of the FGFR, then peptide mimetics of the sequence would also be expected to inhibit N-cadherin and FGFR responses by binding back to the HAV region of the receptor. To test this, we made a short synthetic peptide (N-Ac-IDPVNGQ-NH2) and tested it in the neurite outgrowth assays. The results in Fig. 5A demonstrate that this peptide can inhibit the N-cadherin and FGFR responses with similar efficacy. Again, the peptide had no effect on basal neurite outgrowth over 3T3 monolayers. We also tested a longer version of the peptide primarily in the N-cadherin assay (N-Ac-WLKDIPVNGQI-NH2). This peptide was moderately better than the shorter peptide at inhibiting the N-cadherin response (IC50 = ~60 μM compared with ~100 μM, see Fig. 5B). When we substituted a single isoleucine to alanine (N-Ac-WLKADPVNGQI-NH2) or proline to alanine (N-Ac-WLKIDAVNGQI-NH2), we saw an approximate 3-fold reduction in the efficacy of the longer peptide (IC50 = ~180 μM). Finally, when we tested the double mutant (N-Ac-WLKADAVNGQI-NH2), we found essentially a complete loss of inhibitory activity (Fig. 5B).

The longer peptide also inhibited the FGFR2 response, and this activity was lost with the double mutation (data not shown).

An Antibody Targeted to the ECD4 Motif Inhibits the N-cadherin Response—To further test whether the ECD4 of N-cadherin contains a functional motif required for the neurite outgrowth response, a rabbit antiserum was raised against a synthetic peptide that contains the inhibitory peptide motif (RYTKLSDPANWLKIDPVNGQIT), and an affinity purified fraction was prepared. We used the affinity purified antibody to immunoblot lysates from a variety of cell lines (Fig. 6A). The antibody showed weak reactivity against 3T3 cell lysates, however, a clear band at the appropriate molecular weight was observed in transfected 3T3 cells that express chick N-cadherin (the LK8 cell line). An appropriate band was also detected in the BT-549 and MDA-MB436 tumor cell lines that express human N-cadherin and in COS-7 cells. In contrast, no immunoreactivity was found in cell lines that are known not to express N-cadherin (MCF-7 and MDA-MB231 cells) or in parental or FGFR-expressing L6 cells. Some of the N-cadherin-negative cell lines express E-cadherin and Cadherin-11, and the BT-549 cells express P-cadherin, which runs at a slightly lower molecular weight than does N-cadherin (data not shown, but for details, see Ref. 21). Thus, we can conclude that the antibody reacts specifically with N-cadherin and does not cross-react with E-cadherin, Cadherin-11, P-cadherin, or the FGFR. The affinity purified fraction of the antiserum also showed good reactivity against native N-cadherin as determined by enzyme-linked immunosorbent assay (Fig. 6B) in the absence of any detectable binding to recombinant ECD1 of N-cadherin (data not shown).

The affinity purified antibody was tested for its effects on the neurite outgrowth response stimulated by N-cadherin. When used at 67 ng/ml, the antibody completely inhibited the N-
Table I

The effects of peptide mimetics of FGFR binding sites on the neurite outgrowth response stimulated by FGF and N-cadherin

Neurons were cultured on parental 3T3 monolayers in control medium or medium containing FGF2 (1 ng/ml) or on monolayers of 3T3 cells that express physiological levels of transfected N-cadherin (the LK8 cell line). The medium was then further supplemented with 100 μg/ml peptide mimetic of the given binding motif. In all cases, linear peptide mimetics of the binding motif were synthesized, acetylated, and amidly-blocked (e.g. N-Ac-HFKDPKRLY-NH2 and N-Ac-YCSNGGHFC-NH2). After ~18 hours, the cultures were fixed, and the mean length of the longest neurite was determined by sampling ~120 neurons in replicate wells. The results show the percentage inhibition of the response stimulated by FGF2 (1 ng/ml) and N-cadherin mean ± S.E. from at least three experiments. The peptides had no effect on basal neurite outgrowth over 3T3 monolayers, and typical control responses to N-cadherin and FGF are shown in Figs. 1 and 3. In the case of the 21GNYKKPK27 and 30YCSNGGHF37 motifs, the results obtained with the linear and cyclic peptides were not distinguishable, and these have been pooled for inhibition of the FGF2 response.

| Cluster | Sequence          | Inhibition |
|---------|------------------|------------|
|         |                  | FGF        | Ncad*     |
| 1a      | 21GNYKKPK27      | 91 ± 10    | 12 ± 4.6  |
|         | Cyclic peptide   |            |           |
| 1b      | 25HFKDPKRLY33    | 97 ± 5.5   | 2 ± 7.8   |
| 1c      | 37YCSNGGHFC51    | 65 ± 3.0   | 78 ± 10.4 |
|         | Cyclic peptide   |            |           |
| 2       | 61LSAESVGGEY70   | 76 ± 6.1   | 14 ± 1.4  |
| 3       | 102LRLEENHHN110  | 30 ± 5.2   | 2 ± 2.7   |
| 4       | 148LPLQVSSD155   | 46 ± 5.8   | 10 ± 0.3  |

* Ncad, monolayers of N-cadherin.

DISCUSSION

A large body of evidence suggests that some N-cadherin responses are dependent on FGFR function in cells (Introduction). A key result that supports this conclusion is the observation that when expressed in neurons, a kinase-deleted dominant negative form of the FGFR inhibits the neurite outgrowth response stimulated by N-cadherin. The validity of the conclusions from the dominant negative FGFR experiments has recently been challenged, based on the fact that the remaining cytoplasmic portion of the kinase-deleted receptor still contained a binding site for the adapter molecule FRS2 (35). The recent availability of a highly selective inhibitor of the tyrosine kinase activity of the FGFR (PD 173074) has allowed us to revisit this important question. This compound was found to inhibit the neurite outgrowth response stimulated by N-cadherin and FGF2 with similar efficacy. This compound binds to the ATP-binding pocket of the FGFR (31), and although this pocket is relatively well conserved in closely related tyrosine kinase receptors at the concentration used, this compound has no effect on the insulin-like growth factor, platelet-derived growth factor, nerve growth factor, BDNF, CNTF, GDNF, and epidermal growth factor receptors (31, 32). Given the congruence of the results obtained with the dominant negative FGFR (17) and the PD 173074 compound described in this study, we can conclude that the neurite outgrowth response stimulated by N-cadherin does indeed depend on FGFR activity in neurons. Likewise, neurite outgrowth stimulated by NCAM and L1 are also further established to be dependent on FGFR activity in neurons.

N-cadherin might act as a surrogate ligand for the FGFR and/or sensitize the FGFR to low levels of an FGF present in the cultures. Indeed, using the induction of the matrix metalloproteinase MMP-9 as a read-out, it has been shown that the expression of N-cadherin in the MCF-7 breast cancer cell line leads to a dramatic increase in the sensitivity to FGF2, however, the FGF2 was exogenous rather than endogenous (20). In the context of the neurite outgrowth response stimulated by N-cadherin, all of the evidence suggests that the cultures do not inhibit the N-cadherin response in the absence of any significant effect on the response stimulated by FGF2 (Fig. 7). As an additional control, we also cultured neurons on monolayers of 3T3 fibroblasts that express physiological levels of the NCAM or L1 adhesion molecules, because both of them can also stimulate neurite outgrowth via an FGFR-dependent mechanism (16). The antibody to N-cadherin had no effect on these responses (Fig. 7). To rule out the possibility that the antibody was inhibiting function by clustering N-cadherin in the neurons and perhaps inducing internalization, we prepared a monovalent F(ab')2 fraction from the whole antisera. This reagent substantially inhibited the N-cadherin response in the absence of any effect on basal neurite outgrowth over 3T3 monolayers or neurite outgrowth stimulated by NCAM or L1 (Fig. 7). To determine whether the antibody was inhibiting function by binding to N-cadherin in the neuron and/or the substrate, we pretreated the neurons and/or monolayers with antibody prior to co-culture. The pretreatment of neurons with the monovalent F(ab')2 inhibited the N-cadherin response, whereas the pretreatment of the substratum had no effect (Fig. 8).
contain functional levels of an endogenous FGF (e.g. the FGF antagonists have no effect on basal neurite outgrowth). Nonetheless, the possibility of sensitization to a "subthreshold" level of an FGF is difficult to discount. To try to resolve this issue, we used a structural bioinformatics approach to design a range of novel FGFR antagonists. We reasoned that peptide mimetics of receptor binding motifs might be able to bind back to the receptor and antagonize FGF function. Given that all of the FGFs are thought to interact with common regions that are conserved among all four FGFRs (29), such agents would be expected to act as general FGF antagonists. A total of eight peptide mimetics based on four linear amino acid clusters were shown to be able to inhibit the neurite outgrowth response stimulated by FGF with varying degrees of efficacy, and control experiments pointed to the fact that these peptides were indeed acting at the level of the ligand interacting with the receptor. The peptides that failed to inhibit the N-cadherin response are all mimetics of FGF sequences that are known to interact with the linker region between Ig domains 2 and 3 or with Ig domain 3 of the receptor. This finding suggests that if N-cadherin interacts with the FGFR, then it does so in a manner that does not fully

![Image](http://example.com/image.png)

A Novel Functional Motif in N-cadherin ECD4

**Fig. 3.** The effect of FGFR binding motif peptide mimetics on the neurite outgrowth response stimulated by FGF2. Neurons were cultured on parental 3T3 monolayers in control medium or medium containing FGF2 (1 ng/ml), and the medium was then further supplemented with up to 100 μg/ml a peptide mimetic of the given interacting motif. A, we tested a linear mimetic of the functional motif (N-Ac-HFKDPKRLY-NH2). B, we tested both a linear (N-Ac-GNYYKKPK-NH2) and a cyclic (N-Ac-CGNYKKPKC-NH2) mimetic of the functional motif. C, we tested both a linear (N-Ac-YCSNGGHF-NH2) and cyclic (N-Ac-YCSNGGHFC-NH2) mimetic of the functional motif. D, we tested a linear mimetic of the functional motif (N-Ac-LSAESVGEVY-NH2). After ~18 h, the cultures were fixed, and the mean length of the longest neurite was determined by sampling ~120 neurons in replicate wells. The results show absolute neurite length, and each value is the group mean pooled from 2–5 independent experiments. Bars show ± S.E. in those instances where more than two experiments were performed. In B and C, we have pooled the results with the cyclic and linear peptides, because they did not differ in activity.

**Fig. 4.** A peptide that "targets" the HAV region of the FGFR inhibit the neurite outgrowth response stimulated by N-cadherin. A, neurons were cultured on monolayers of control 3T3 cells or monolayers of N-cadherin expressing 3T3 cells in medium supplemented with the N-Ac-YCSNGGHF-NH2 peptide as indicated. This peptide had no effect on basal neurite outgrowth but inhibited the N-cadherin response in a dose-dependent manner. Results show the mean neurite length (± S.E.) from 4–6 independent experiments. B, we analyzed FGF1/FGFR2 and FGF1/FGFR1 crystals (see under "Experimental Procedures" for details) to identify the receptor binding sites for the YCSNGGHF motif in FGF1. These sites are given as the inserts.
overlap with the binding sites for FGFs. Interestingly, two peptide mimetics of a small motif in FGF1 (30YCSNGGHF37) were as effective at inhibiting the N-cadherin response as the FGF2 response. An analysis of crystal structures shows that this sequence within FGF1 and the homologous sequence in FGF2 make extensive and relatively exclusive contacts with the HAV region of the FGFRs. Interestingly, other data support a function for this region of the FGFR in N-cadherin responses. For example, antibodies directed to this site on the receptor and peptide mimetics of this region of the receptor both inhibit the neurite outgrowth response stimulated by N-cadherin (16).

Thus, if the inhibitory peptides are acting as true mimetics of the 30YCSNGGHF37 motif, they most probably inhibit both FGF and N-cadherin function by binding to the HAV region of the FGFR.

The IDPVNGQ motif from ECD4 of N-cadherin has previously been identified as a candidate binding motif for the HAV region of the FGFR based on sequence homology with the motifs within N-cadherin (INPISGQ) and R-cadherin (IDPVSGR) that interact with the HAV region of N-cadherin (22, 38). To determine whether the ECD4 motif has the potential to interact with the FGFR we initially adopted a peptide competition approach, as the clear prediction is that peptide mimetics based on this motif should be able to inhibit both FGF and N-cadherin responses. We found that a long peptide mimetic (N-Ac-WLKIDPVNGQI-NH2) and short peptide mimetic (N-Ac-IDPVNGQ-NH2) could both inhibit the neurite outgrowth responses stimulated by N-cadherin and FGF. The fact that the cadherin-derived peptide can antagonize the FGF response provides evidence, albeit indirect, that this motif can interact with the FGFR.

To further test the function of the candidate FGFR binding site in ECD4 of N-cadherin, an antibody was raised against a peptide (RYTKLSDPANWLKIDPVNGQIT) that contained the putative binding motif. The antibody was fully characterized and shown to be specific to N-cadherin. An affinity purified fraction of the antiserum was shown to fully inhibit the N-cadherin response in the absence of any effect on an FGF2 response or an NCAM and L1 response. Furthermore, a monovalent F(ab’)/H11032 fraction of the antiserum also specifically inhibited the N-cadherin response. The possibility of the antibody inhibiting cadherin function by inhibiting homophilic binding is improbable for a number of reasons. Firstly, recent experimental evidence on N-cadherin-stimulated neurite outgrowth (38, 39) concurs with the structural evidence that points to a direct interaction between the ECD1 domains as mediating the

**FIG. 5.** Peptide mimetics of an N-cadherin ECD4 motif inhibit the neurite outgrowth response stimulated by FGF and N-cadherin. Cerebellar neurons were cultured on 3T3 monolayers in control medium or medium containing 1 ng/ml FGF2 or on monolayers of 3T3 cells expressing N-cadherin. We evaluated the ability of five peptides to inhibit the FGF and/or N-cadherin responses. A, the effect of the N-Ac-IDPVNGQ-NH2 peptide on the N-cadherin and FGF response is shown. B, the effect of the N-Ac-WLKIDPVNGQI-NH2 peptide and three “mutated” versions of this peptide on the N-cadherin response is shown. The sequence of the I/A peptide was N-Ac-WLKADPVNGQI-NH2. The sequence of the P/A peptide was N-Ac-WLKDADVNGQI-NH2, and the sequence of the I/A and P/A peptide was N-Ac-WLKDADVNGQI-NH2.

The results show the percent inhibition of the neurite outgrowth response stimulated by N-cadherin and FGF2 (typical control responses are shown in Figs. 1 and 3), and each value is the mean ± S.E. determined from three independent experiments. None of the peptides had any significant effect on the basal neurite outgrowth over parental 3T3 monolayers (data not shown).

**FIG. 6.** Specificity of the N-cadherin ECD4 peptide antiserum. A, lysates from a variety of cell types as indicated were resolved by SDS-polyacrylamide gel electrophoresis and immunoblotted with the affinity purified fraction of the antiserum (1/1000 dilution of stock) raised against the N-cadherin ECD4 peptide. B, the affinity purified antiserum tested for reactivity to native N-cadherin by enzyme-linked immunosorbent assay titration against an N-cadherin-Fc chimera.
homophilic binding interaction (26, 40). Secondly, our antibody only inhibits function when bound to N-cadherin in the neurons (as compared with the substrate), and this fact is difficult to reconcile with the antibody inhibiting homophilic binding. Finally, it has recently been reported that when a 69-amino acid sequence from ECD4 of N-cadherin, which contains the above motif, is swapped with the corresponding segment of E-cadherin, the new chimeric version of E-cadherin acquires the ability of N-cadherin to promote the migration of a cancer cell line (41). An antibody that interacts specifically with the N-cadherin sequence and has no effect on N-cadherin-mediated adhesion was able to fully inhibit the motility response. Thus, it appears probable that the ECD4 contains a motility motif that can function independently from the homophilic binding site. In terms of the axonal growth response, our data point to this motif mediating a functional interaction with the FGFR.

What mechanism might account for an N-cadherin reliance on FGFR function but not FGF function? The answer to this question might be related to the fact that FGFRs can be activated in a ligand-independent manner simply by overexpressing them in cells (42). In this context, when N-cadherin is clustered with antibodies, the FGFR is found concentrated in the clusters (15). Furthermore, N-cadherin co-immunoprecipitates with the FGFR in ovarian cells (23). Interestingly, in pancreatic tumor cells the co-precipitation of N-cadherin with the FGFR has been reported to be dependent on NCAM expression in the cells (24), however, we have found co-precipitation among these molecules in cells that do not express NCAM. Nonetheless, we have as yet been unable to inhibit the co-immunoprecipitation of N-cadherin and the ECD4 antibody, one possible explanation being that additional sites and/or molecules contribute to the interaction. Irrespective of whether the N-cadherin interacts directly with the FGFR or indirectly as part of a larger complex, cadherin homophilic binding and the consequent lateral dimerization (40) provide a basis for the relocalization of the FGFR in the cell membrane and its consequent activation in a manner that need not rely on the function of the more conventional receptor ligands.

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FIG. 7. The effects of an N-cadherin ECD4 antiserum on the neurite outgrowth responses stimulated by N-cadherin, FGF2, NCAM, or L1. Cerebellar neurons were cultured for 18 h on monolayers of 3T3 cells in control medium or medium containing 1 ng/ml FGF2 or on monolayers of 3T3 cells expressing N-cadherin, NCAM, or L1 (as indicated by the filled bars). Sister cultures were supplemented with 67 ng/ml affinity purified polyclonal antibody to ECD4 of N-cadherin (hatched bars) or with 80 μg/ml monovalent F(ab′)’ fraction prepared from the whole serum (open bars). In the case of the 3T3, N-cadherin, and FGF2 responses, the results show the mean length of the longest neurite/cell pooled from three independent experiments. In the case of the NCAM and L1 responses, the results are from a single representative experiment. The bars show the mean ± S.E. for the three independent experiments or for the population of neurons from the single experiment.

FIG. 8. The ECD4 antiserum acts on the neuron. Cerebellar neurons were either left in control medium (filled bars) or treated with 80 μg/ml F(ab′) fraction of the N-cadherin-ECD4 antibody for 1 h at 37 °C before being washed and cultured on monolayers of parental 3T3 cells or monolayers expressing N-cadherin (hatched bars). Alternatively, the control and N-cadherin expressing monolayers were treated with 80 μg/ml F(ab′) fraction antibody for 1 h at 37 °C before three washes with control medium and the subsequent addition of untreated cerebellar neurons (open bars). After 18 h, the cultures were fixed, and the mean neurite length was determined. The results show that a pretreatment of neurons, but not substrate, with the F(ab′) fraction of the N-cadherin-ECD4 antibody is sufficient to inhibit the neurite outgrowth response stimulated by N-cadherin. The results show the absolute neurite length; each value is the mean ± S.E. measured from 100–150 neurons sampled from replicate cultures.
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