Dimeric Versions of Two Short N-cadherin Binding Motifs (HAVDI and INPISG) Function as N-cadherin Agonists*

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N-cadherin is a member of the classical cadherin family of homophilic binding molecules. Peptide competition studies have identified the HAVDI and INPISGQ sequences as functional binding motifs in extracellular domain 1 (ECD1) of N-cadherin. Whereas monomeric versions of these motifs function as specific N-cadherin antagonists, we now show that cyclic peptides containing a tandem repeat of the individual motifs function as N-cadherin agonists. In this context, when presented to neurons as soluble molecules, the dimeric versions of the motifs stimulate neurite outgrowth in a similar manner to native N-cadherin. The response to the dimeric agonist peptides was inhibited by monomeric versions of the same motif and also by recombinant N-cadherin ECD1 protein. The responses were also inhibited by antibodies to a fibroblast growth factor receptor (FGFR) binding motif in ECD4 of N-cadherin and by a specific FGFR antagonist (PD17304). These data suggest that the peptides function by binding to and clustering N-cadherin in neurons and thereby activating an N-cadherin/FGFR signaling cascade. The novel agonists will be invaluable for dissecting out those cadherin functions that rely on signaling as opposed to adhesion and clearly have the potential to be developed as therapeutic agents for the promotion of cell survival and axonal regeneration.

The transmembrane glycoproteins of the classical cadherin family are homophilic binding molecules that mediate cellular recognition in numerous developmental contexts (1). They underpin the formation of stable adhesive connections between cells, and this requires indirect binding to the actin cytoskeleton (2). In some developmental situations, cadherins promote cell survival (3, 4), cell migration (5), axonal growth (6), and synaptic plasticity (7, 8), with some of these functions most probably depending upon the activation of intracellular signal transduction cascades in cells.

In this context of cell signaling, regulated tyrosine phosphorylation is important for both the classical adhesive (9) and non-adhesive (10) functions of cadherins. A functional interaction between a receptor tyrosine kinase and a cadherin was initially suggested based on the observation that neurite outgrowth stimulated by N-cadherin requires the activity of the fibroblast growth factor receptor (FGFR) in neurons (11). It has now been shown that N-cadherin and the FGFR associate with each other in several cell types (12–14). More recently, a novel functional motif that interacts with the FGFR has been mapped to extracellular domain 4 (ECD4) of N-cadherin (15). Direct and/or indirect cadherin interactions with a wide range of receptor tyrosine kinases, including the epidermal growth factor receptor (16), the c-Met receptor (17), and the Ephrin A2 receptor (18), have also been reported. Likewise, cadherins can interact with both receptor and non-receptor tyrosine phosphatases, with protein-tyrosine phosphatase-1β and -μ binding sites recently mapped to the cadherin cytoplasmic domain (19, 20). In principle, regulated tyrosine phosphorylation could act upstream of the homophilic recognition step to modulate the prevalence, localization, or activation state of the cadherin in the cell membrane. Alternatively, regulated tyrosine phosphorylation might be a consequence of homophilic recognition and, under these circumstances, might serve to couple this event to a cellular response.

In terms of the functional consequences of cadherins interacting with receptor tyrosine kinases, an interaction between N-cadherin and the FGFR has been implicated in both developmental and pathological processes. For example, neurite outgrowth stimulated by N-cadherin is inhibited by a wide variety of agents that inhibit FGFR function in neurons (11), including the expression of a dominant-negative FGFR (12, 21, 22) and a recently developed FGFR antagonist (15). In addition, N-cadherin can promote “contact-dependent” survival of ovarian granulosa cells in an FGFR-dependent manner (23). 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 (24, 25). Whereas antagonists of N-cadherin have obvious therapeutic potential in terms of cancer, agonists have the potential to be developed as therapeutic agents that might promote cell survival and/or axonal regeneration.

The fact that soluble forms of some adhesion molecules (26), including N-cadherin (12), can promote axonal growth as effectively as membrane-tethered forms suggests that the development of small molecule agonists might be an attainable goal. In principle, this might be accomplished by using dimeric mimetics of natural binding motifs, as these would have the potential to dimerize cadherins (and any associated signaling molecules) in the cell membrane. In this context, the extracellular portions of the classical cadherins are composed of five repetitive domains, and a large body of evidence suggests that the homophilic binding site resides in the amino-terminal domain.
The fact that peptide mimetics of two linear sequences from ECD1 (HAVDI and INPISGQ) function as highly specific N-cadherin antagonists in a physiologically relevant assay (29, 30) is consistent with the view that this domain contains key binding motifs. In this study, we have designed cyclic peptides that contain tandem mimetics of the above binding motifs. Our results show that, like native N-cadherin, these peptides can promote neurite outgrowth from cultured cerebellar neurons. Moreover, we show that, as with the response to native N-cadherin, the response to the peptide antagonists requires the function of both N-cadherin and the FGFR in the responding neuron.

**EXPERIMENTAL PROCEDURES**

**Neurite Outgrowth Assays—**Co-cultures of cerebellar neurons on monolayers of parental 3T3 cells or an established transfected 3T3 cell line that expresses physiological levels of chick N-cadherin (the LK5 cell line; see Ref. 31 for details) were established as previously described (11). These cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. To establish the co-cultures, ~80,000 3T3 cells (or LK5 cells) were plated into individual chambers of an eight-chamber tissue culture slide coated with poly-lysine and fibronectin and maintained overnight in Dulbecco's modified Eagle's medium and 10% fetal calf serum to allow for the formation of a confluent monolayer. The medium was removed, and ~6000 dissociated cerebellar neurons (taken from postnatal day 3 rats) were plated into each well in SATO medium supplemented with 2% fetal calf serum. Test reagents were added as indicated below, and the co-cultures were maintained for 18 h. The co-cultured neurons were fixed and stained for GAP43 immunoreactivity. The mean length of the longest neurite per cell was measured for ~120–150 neurons as previously described (11).

**Anti-N-cadherin ECD4 Antibody and the FGFR Antagonist—**A rabbit antiserum was affinity-purified against the peptide immunogen and functional motif that can interact with the FGFR in neurons (15). The antisera was affinity-purified against the peptide immunogen and stored as a stock at 40 µg/ml in phosphate-buffered saline/glycerol (50:50) at ~20 °C. A monovalent (F(ab′)2 fraction of the whole antiserum was also prepared by standard methods, purified by HPLC, and stored as a stock at 33 mg/ml at 4 °C. The FGFR antagonist PD17304 (32) was synthesized as previously described (33, 34).

**N-cadherin Structures—**For the purposes of molecular modeling, we primarily made use of the trans-adhesion crystal dimer of ECD1 of N-cadherin (Protein Data Bank code 1NCH) (35). Molecular Simulations, Inc. and Swiss Protein Database software packages were used to isolate the structure of the HAVDI and INPISGQ motifs from the adhesion interface of the crystal. It should be noted that the structure of these motifs is invariant in a number of independent N-cadherin crystals.

**N-cadherin ECD1 Protein—**ECD1 of N-cadherin was in the form of a recombinant protein and was a kind gift from Dr. David Colman. It was stored in small aliquots at 20 mg/ml in phosphate-buffered saline at ~20 °C. Full details of the construct and methods for preparing the recombinant protein can be found in Ref. 35.

**Peptide Synthesis and Purity—**Synthetic peptides were primarily obtained from a commercial supplier (Multiple Peptide Systems). The N-Ac-CHAVDIC-NH2 and N-Ac-INPISGQ-NH2 peptides were previously described (29, 30) and were a kind gift from Drs. Barbara Gour and P. N. Mahesh (Adherex Inc.) All peptides were purified by reverse-phase HPLC and obtained at the highest level of purity (≥95%). An underlined peptide sequence denotes a peptide that has been cyclized via a disulfide bond between the given cysteine residues.

**Surface Plasmon Resonance Measurements—**Surface plasmon resonance experiments were performed with a BIAcore XSM biosensor (Amersham Biosciences, Inc., Uppsala, Sweden). Here, recombinant N-cadherin ECD1 protein was immobilized on one flow cell of a CM5 chip via amine coupling according to the manufacturer's protocol; the other flow cell served as a control. The recombinant ECD1 protein was diluted into the manufacturer's running buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 3.4 mM EDTA, and 0.005% P2O) to concentrations of 6.6, 2.8, 1.4, and 0.7 µM and passed over the chip at a flow rate of 10 µl/min for a period of 3 min. This resulted in a consistent set of binding curves that demonstrate the homophilic association of soluble ECD1 with immobilized ECD1.

**RESULTS**

**A Dimeric Version of the HAVDI Motif Promotes Neurite Outgrowth—**Peptide mimetics of the HAVDI motif in ECD1 of N-cadherin function as specific N-cadherin antagonists (30), suggesting that this sequence contains a functional N-cadherin binding motif. This view is supported by the presence of the sequence at the interface of the ECD1 adhesion dimer crystal (35), where side chains from this sequence account for ~14% of the adhesion interface. We designed a cyclic peptide that contained the HAVDI sequence in tandem (N-Ac-CHAVDINGHAVDIC-NH2). To ensure that both HAVDI motifs have the potential to adopt the natural structure of this sequence (as deduced from an examination of the ECD1 adhesion dimer interface revealed in the 1NCH crystal), we included the native asparagine and glycine residues as a linker between the motifs (see Fig. 1 for details). Molecular modeling also clearly demonstrated that the cyclic peptide has the potential to simultaneously bind to two cadherin molecules in the plane of the same membrane and therefore has the potential to dimerize N-cadherin in a cis-configuration.

**Fig. 1. The model structure of the dimeric HAVDI peptide.** The native structure of the HAVDI sequence was isolated from the crystal of the intact ECD1 protein of N-cadherin (Protein Data Bank code 1NCH). This is shown above and below a possible structure for the cyclic N-Ac-CHAVDINGHAVDIC-NH2 peptide (upper). The disulfide bond in the cyclic peptide is shown in yellow. The composite peptide image is shown in stereo for extra clarity. This analysis shows that the cyclic peptide has the potential to adopt a structure that would allow for the presentation of both HAVDI binding motifs in a natural configuration and in an antiparallel manner. Taking the model cyclic peptide structure and assuming that each HAVDI motif docks with ECD1 in the same way as the native sequence in the trans-adhesion dimer crystal (code 1NCH), we can construct a model for ECD1 dimerization by the cyclic peptide (lower). This model demonstrates that the peptide has the potential to simultaneously bind to two cadherin molecules in the plane of the same membrane and therefore has the potential to dimerize N-cadherin in a cis-configuration.
experiments, physiological levels of transfected N-cadherin expressed in the LK8 cell line stimulated neurite outgrowth to 174.5 ± 9.5% of the control 3T3 cell value (mean ± S.E. from five independent experiments). Thus, the responses to native N-cadherin and the dimeric peptide are similar in magnitude. When a maximally active concentration of the peptide was added to neurons growing over the transfected N-cadherin-expressing cells, there was no further stimulation of growth (data not shown), demonstrating that the effects were nonadditive. When the dimeric HAVDI motif was presented to neurons as a linear rather than a cyclic peptide (N-Ac-HAVDINGHAVDI-NH₂), it had no effect on neurite outgrowth (Fig. 2). These data demonstrate that the N-Ac-HAVDINGHAVDI-NH₂ peptide can promote neurite outgrowth and that this activity is dependent on the peptide being cyclic.

A Dimeric Version of the INPISG Motif Promotes Neurite Outgrowth—Peptide mimetics of the N-cadherin ECD1 INPISGQ sequence also function as highly effective and specific N-cadherin antagonists (29), suggesting that this linear sequence contains a second functional binding motif. We designed a cyclic peptide that contained the INPISG sequence in tandem (N-Ac-CINPISGINPISGC-NH₂), taking care to ensure that both INPISG motifs have the potential to adopt the natural structure of this sequence as determined from the N-cadherin ECD1 adhesion dimer crystal (code 1NCH). We can construct a model for ECD1 dimerization by the cyclic peptide (lower). This model demonstrates that the peptide has the potential to simultaneously bind to two cadherin molecules in the plane of the same membrane and therefore has the potential to dimerize N-cadherin in a cis-configuration.

Monomeric Peptides Inhibit the Response to the Dimeric Agonist Peptides—Monomeric mimetics of the INPISGQ sequence function as specific N-cadherin antagonists (29). For example, when used at 100 μg/ml, the linear N-Ac-INPISGQ-NH₂ peptide inhibits the neurite outgrowth response stimulated by native N-cadherin in the absence of any significant effect on the neurite outgrowth response stimulated by NCAM, L1, or FGF2 (29). The data in Fig. 5 show that this monomeric peptide mimetic of the INPISGQ sequence could completely inhibit the effect of a maximally active concentration of the dimeric INPISG agonist peptide. Likewise, monomeric mimetics of the HAVDI motif also function as highly specific N-cadherin antagonists; however, in this case, cyclic peptide mimetics have greater efficacy (30). The data in Fig. 5 show that when added
Experimental Procedures

ECD1 of N-cadherin over a BIAcore sensor chip that had the peptide immobilized N-cadherin ECD1 (data not shown). These data show that homophilic binding between ECD1 domains of the intact ECD1 protein of N-cadherin and the FGFR in neurons (15). The effects of a monovalent F(ab') fraction of this rabbit antisera on the response to the peptide agonists were determined in the coculture assay. At 80 μg/ml, the monovalent F(ab') antibody completely inhibited the neurite outgrowth response stimulated by the cyclic dimeric HAVDI and INPISG agonist peptides (Fig. 8). Under the same conditions, this antibody inhibits the response to native N-cadherin in the absence of any effect on the neurite outgrowth response stimulated by FGF2, NCAM, and L1 (15). The conclusion that N-cadherin-dependent neurite outgrowth requires FGFR function in neurons has recently been further substantiated by showing that a highly specific antagonist of the FGFR (PD17304) (32, 33) can inhibit the neurite outgrowth response stimulated by FGF2 and native N-cadherin with similar efficacy (15). This reagent also fully inhibited the response stimulated by the cyclic dimeric HAVDI and INPISG agonist peptides (Fig. 8). Thus, we can conclude that the dimeric peptide agonists also stimulate neurite outgrowth via an FGFR-dependent mechanism.

DISCUSSION

Much of the function of the classical cadherins depends on their ability to promote the stable adhesion of cells to each other. However, it is becoming apparent that simple adhesion models cannot readily explain the diverse functions of some cadherins. For example, N-cadherin has been implicated in a number of developmental events that range from promoting cell survival (3, 4) to controlling axonal growth, guidance, synapse formation, and synaptic plasticity (6–8, 37–39). Given our broader understanding of the function of adhesion molecules such as NCAM and L1 (40), it appears probable that some cadherin functions might be explained by their ability to activate signal transduction cascades in cells rather than by adhesion per se.

One cadherin function that might be best explained by recognition and signal transduction is N-cadherin-stimulated neurite outgrowth. Evidence for this includes the observation that a soluble dimeric form of N-cadherin can stimulate neurite outgrowth as effectively as N-cadherin expressed in a transfected cell line (12). In principle, whereas a monomeric mimic of a key binding motif might be expected to function as an antagonist, a dimeric version of the motif might have the potential to be developed as an agonist. In this context, putative binding sites within ECD1 of N-cadherin were originally identified based on peptide competition assays (31, 41). More recently, peptide mimetics of the INPISQ and HAVDI sequences have been shown to inhibit N-cadherin-stimulated neurite outgrowth, with IC_{50} values of ~15 and 65 μM, respectively (29, 30). Based on these observations, we conclude that ECD1 of N-cadherin contains at least two short linear binding sequences that contribute to the homophilic recognition process that leads to a neurite outgrowth response.
FIG. 6. Homophilic binding of N-cadherin ECD1. Soluble recombinant ECD1 of N-cadherin was passed over a flow chip coated with the same protein for 180 s, at which time the chip was rapidly washed with running buffer on its own. The ECD1/ECD1 association/dissociation curves are shown plotted in resonance units with background binding to the control well of the flow cell subtracted. The curves are for ECD1 concentrations of 0.6, 2.8, 1.4, and 0.7 μM and show a clear dose dependence. The inset is a plot of $k_{\text{obs}} = k_a C + k_d$, where $k_a$ and $k_d$ are the association and dissociation constants, respectively, and $C$ is the concentration against the ECD1 concentration, demonstrating a linear dose dependence with a linear regression correlation coefficient of 0.97.

Using a BIAcore approach, we have shown that ECD1 of N-cadherin can interact with itself. We have also shown that the recombinant ECD1 protein functions as a very active and specific N-cadherin antagonist, with an IC$_{50}$ of <5 nM. The fact that the BIAcore interaction was best seen at protein concentrations that are higher than those required for inhibition of neurite outgrowth might be expected given the disordered nature of ECD1 when coated on a BIAcore chip relative to the ordered nature of N-cadherin in a cell membrane. Colman and co-workers (35) have shown that the same recombinant ECD1 protein will form two distinct crystal dimers; one has the characteristics of a trans-adhesion dimer (Protein Data Bank code 1NCH), and the second has the characteristics of a cis-dimer (code 1NCG). Interestingly, the HAVDI and INPISGQ sequences are both present at the trans-adhesion dimer interface revealed in the 1NCH crystal, where they interact in a reciprocal manner. Based on the above observations, the most parsimonious explanation of our data is that the peptide mimetics of these sequences function as antagonists by competing for natural binding sites that are present in ECD1 of N-cadherin. In support of this, molecular modeling has demonstrated that the antagonist peptides can adopt the same structure as the natural motifs present at the adhesion dimer interface that is formed between two ECD1 monomers (29, 30). Structural and biochemical studies point to additional ways in which classical cadherins might form both cis- and trans-dimers (28), and two recent reports have suggested that cadherin-mediated adhesion might involve an overlap of all the cadherin domains (36, 42). It is perhaps worth noting that we have been unable to inhibit the binding of a neuronal cell line to purified N-cadherin using the above peptides at relatively high concentrations. This tends to support the view that there might be fundamental differences in the way that cadherins interact with each other in a transient manner as opposed to a stable manner, and this might explain in part the lack of congruence in the structural and biochemical studies. Nonetheless, our studies with peptide antagonists lend support to the view that the trans-adhesion interface revealed in the 1NCH crystal dimer is a biologically relevant interface, albeit with the caveat that our studies speak only to the homophilic recognition that promotes an axonal growth response.

If the dimeric versions of the above peptide antagonists can bind to their “natural” binding sites as revealed in the trans-crystal dimer, they might be expected to promote the dimerization of cadherins in the cell. In principle, the peptides might dimerize cadherin monomers and/or pre-existing cis-dimers, as the cis-dimerization face is on the opposite side of the molecule to the proposed peptide binding sites (35). Based on the above hypothesis, we have tested dimeric forms of both the HAVDI and INPISG sequences for their ability to function as N-cadherin agonists. We designed peptides that contained the motifs in an antiparallel manner, as our molecular modeling studies suggested that this orientation would be required for the simultaneous engagement of two cadherin units. Our results have shown that cyclic peptides containing dimeric HAVDI and INPISG motifs (N-Ac-CHAVDINGHAVDIC-NH$_2$ and N-Ac-CINPISGINPISGC-NH$_2$) can promote neurite outgrowth; and in the case of the HAVDI agonist peptide, the response was almost as good as that stimulated by native N-cadherin. As a control, we have shown that when both peptides are presented to neurons as linear rather than cyclic peptides, they do not stimulate neurite outgrowth. This was to be expected given the fact that the molecular modeling showed that the disulfide bond within each active peptide can constrain the structure in a manner that would be expected to facilitate the interaction of one peptide with two cadherin molecules. The observation that the response to the peptides is biphasic might be explained by monomeric binding dominating dimeric binding at higher peptide concentrations. However, it might also be related to the fact that the FGFR signal transduction cascade (which is required for the peptide response; see below) is biphasic, with higher levels of activation inhibiting neurite outgrowth (45, 44).

Four lines of evidence suggest that an interaction with N-cadherin is required for the neurite outgrowth response stimulated by the dimeric HAVDI and INPISGQ peptides. First, the response to each peptide is inhibited by monomeric mimetics of the corresponding motif, and these have previously been established to function as specific N-cadherin antagonists (29, 30). Second, the recombinant ECD1 protein of N-cadherin inhibits the agonist peptide responses, suggesting that they share a common cellular binding site. In this context, a considerable body of evidence points to ECD1 of cellular N-cadherin as the best candidate binding site for the recombinant protein (27), and it follows that this must also be considered as the best candidate binding site for the agonist peptides. Given the molar

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\[2\] F. V. Howell and P. Doherty, unpublished data.
Novel N-cadherin Agonists

FIG. 7. N-cadherin ECD1 functions as an antagonist. In A, cerebellar neurons were cultured over monolayers of control 3T3 cells or monolayers of transfected 3T3 cells expressing physiological levels of N-cadherin (NCAD). Soluble recombinant ECD1 of N-cadherin was included in the media at the given concentrations. After ~18 h, the cultures were fixed, and the mean length of the longest neurite was determined from ~120 neurons sampled from replicate cultures. The results are from a representative experiment and show absolute neurite length, and the bars show the S.E. In B, the effects of recombinant N-cadherin ECD1 protein (30 nM) on the neurite outgrowth response stimulated by the N-Ac-CINPISGINPISG-NH₂ peptide (33 μg/ml) and the N-Ac-CHAVDINGHAVDIC-NH₂ peptide (11 μg/ml) were determined as described in the legend to Fig. 2. The results show the percentage increase in mean neurite length above the control, and each value is the mean ± S.E. from three independent experiments. When added on its own, the anti-ECD4 antibody and the PD17304 antagonist had no effect on basal neurite outgrowth (not shown).

In summary, we have demonstrated the feasibility of the rational design of small molecule N-cadherin agonists. In doing so, we have provided further evidence that the HAVDI and INPISG motifs in ECD1 of N-cadherin are functional binding motifs. There appears to be no reason why the same rationale cannot be employed for the design of other cadherin agonists. The novel reagents will be invaluable tools for dissecting out those cadherin functions that rely solely on signaling from receptors (32, 33). The possibility that the agonist peptides act directly at the level of the FGFR can be discounted, as the response to FGF2 is not inhibited by several of the agents that inhibit the peptide response (e.g. the anti-N-cadherin ECD4 antibody).

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REFERENCES
1. Takeichi, M. (1995) Curr. Opin. Cell Biol. 7, 619–627
2. Gumbiner, B. M. (2000) J. Cell Biol. 148, 399–404
3. Hermiston, M. L., and Gordon, J. I. (1995) J. Cell Biol. 129, 489–506
4. Peluso, J. J., Pappalardo, A., and Trolle, M. P. (1996) Endocrinology 137, 1196–1203
5. Barami, K., Kirschchenbaum, B., Lemmon, V., and Goldman, S. A. (1994) Neuron 13, 567–582

ratio of the peptides to the soluble ECD1 protein (~300:1), we can conclude that ECD1 competes for peptide binding to a cellular site as opposed to acting as a soluble sink for the peptides in solution. The absolute site of interaction with the peptides would clearly be best resolved by direct observation (e.g. crystallography). However, the interaction affinity is likely to preclude this. Nonetheless, synthetic peptides can be viewed as a starting point for the development of non-peptide mimetics, with the latter being much more useful tools for the direct binding studies. Third, the neurite outgrowth response to each agonist peptide is inhibited by a monovalent F(ab') fraction of an antiserum that reacts specifically with a small motif in ECD4 of N-cadherin. This reagent inhibits the neurite outgrowth response stimulated by native N-cadherin in the absence of any effect on neurite outgrowth stimulated by a range of other molecules (15). Recent evidence suggests that the site in ECD4 that is targeted by the antibody plays a role in a cis-interaction between N-cadherin and the FGFR rather than a direct role in homophilic binding (15). The observation that this antibody inhibits the response to peptides that presumably bind to ECD1 provides additional evidence that ECD4 has a function that can be dissociated from conventional homophilic binding (45). Fourth, neurite outgrowth stimulated by native N-cadherin requires FGFR function in the neuron (11, 21, 22). We have shown that a highly specific FGFR antagonist (PD17304) inhibits the agonist peptide responses. It is perhaps worth noting that, at the concentration used, this inhibitor fully blocks the response stimulated by FGF2 and native N-cadherin (15) in the absence of any effect on the insulin-like growth factor, platelet-derived growth factor, nerve growth factor, BDNF, CNTF, GDNF, and epidermal growth factor receptors (32, 33). The possibility that the agonist peptides act directly at the level of the FGFR can be discounted, as the response to FGF2 is not inhibited by several of the agents that inhibit the peptide response (e.g. the anti-N-cadherin ECD4 antibody).
Novel N-cadherin Agonists

6. Matsunaga, M., Hatta, K., Nagafuchi, A., and Takeichi, M. (1988) Nature **334**, 62–64

7. Tsung, L., Huang, C. P., and Schuman, E. M. (1998) Neuron **20**, 1165–1175

8. Bozdagi, O., Shan, W., Tanaka, H., Benson, D. L., and Huntley, G. W. (2000) *Neuron* **28**, 245–259

9. Matusyoshi, N., Hamaguchi, M., Taniguchi, S., Nagafuchi, A., Tsukita, S., and Takeichi, M. (1992) *J. Cell Biol.* **118**, 703–714

10. Williams, E.-J., Walsh, F. S., and Doherty, P. (1994) *J. Cell Biol.* **124**, 1029–1037

11. Williams, E.-J., Furness, J., Walsh, F. S., and Doherty, P. (1994) *Neuron* **13**, 583–594

12. Utton, M. A., Eickholt, B., Howell, F. V., Wallis, J., and Doherty, P. (2001) *J. Cell Biol.* **150**, 1421–1430

13. Peluso, J. J. (2000) *Biochim. Biophys. Acta* **1482**, 137–148

14. Cavallaro, U., Niedermeyer, J., Fuxa, M., and Christofori, G. (2001) *Nat. Cell Biol.* **3**, 650–657

15. Williams, E.-J., Williams, G., Howell, F. V., Skaper, S. T., Walsh, F. S., and Doherty, P. (2001) *J. Biol. Chem.* **276**, 43879–43886

16. Balsamo, J., Arregui, C., Leung, T., and Lilien, J. (1998) *J. Cell Biol.* **147**, 107–116

17. Hiscox, S., and Jiang, W. G. (1999) *Biochem. Biophys. Res. Commun.* **261**, 406–411

18. Zantek, N. D., Azimi, M., Fedor-Chaiken, M., Wang, B., Brackenbury, R., and Kinch, M. S. (1999) *Cell Growth Differ.* **10**, 629–638

19. Balsamo, J., Bozic, D., Pertz, O., and Engel, J. (1999) *J. Cell Biol.* **145**, 523–532

20. Brady-Kalnay, S. M., Mournat, T., Nixon, J. P., Pietz, G. E., Kinch, M., Chen, H., Brackenbury, R., Rimm, D. L., Del Vecchio, R. L., and Tonks, N. K. (1999) *J. Cell Biol.* **141**, 287–296

21. Suzuki, N., Williams, E.-J., Mason, I. J., Walsh, F. S., and Doherty, P. (1997) *Neuron* **18**, 231–242

22. Lom, B., Hopker, V., McFarlane, S., Bixby, J. L., and Holt, C. E. (1998) *J. Neurobiol.* **37**, 633–641

23. Trolice, M. P., Pappalardo, A., and Peluso, J. J. (1997) *Endocrinology* **138**, 107–113

24. Hazan, R. B., Phillips, G. R., Qiao, R. F., Norton, L., and Aaronson, S. A. (2000) *J. Cell Biol.* **148**, 779–789

25. Nieman, M. T., Prudoff, R. S., Johnson, K. R., and Wheelock, M. J. (1999) *J. Cell Biol.* **147**, 631–644

26. Doherty, P., Williams, E., and Walsh, F. S. (1995) *Neuron* **14**, 57–66

27. Shan, W. S., Koch, A., Murray, J., Colman, D. R., and Shapiro, L. (1999) *Biophys. Chem.* **82**, 157–163

28. Koch, A. W., Bozic, D., Pietz, G., and Engel, J. (1999) *Curr. Opin. Struct. Biol.* **9**, 275–281

29. Williams, E.-J., Williams, G., Gour, B., Blaschuk, O., and Doherty, P. (2000) *Mol. Cell. Neurosci.* **15**, 456–464

30. Williams, E., Williams, G., Gour, B. J., Blaschuk, O. W., and Doherty, P. (2000) *J. Biol. Chem.* **275**, 4007–4012

31. Williams, E.-J., Furness, J., Walsh, F. S., and Doherty, P. (1994) *Neuron* **13**, 583–594

32. Mohammadi, M., Froum, S., Hamby, J. M., Schroeder, M. C., Panek, R. L., Lu, G. H., Eliseenkova, A. V., Green, D., Schlessinger, J., and Hubbard, S. R. (1996) *EMBO J.* **15**, 5896–5904

33. Skaper, S. D., Kee, W. J., Facci, L., Macdonald, G., Doherty, P., and Walsh, F. S. (2000) *J. Neurochem.* **75**, 1520–1527

34. Hamby, J. M., Connolly, C. J., Schroeder, M. C., Winters, R. T., Showalter, H. D., Panek, R. L., Major, T. C., Olsewski, B., Ryan, M. J., Dahrin, T., Lu, G. H., Keiser, J., Amar, A., Shen, C., Kraker, A. J., Slintak, V., Nelson, J. M., Fry, D. W., Bradford, L., Hallak, H., and Doherty, A. M. (1997) *J. Med. Chem.* **40**, 2296–2303

35. Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S., Grubel, G., Legrand, J. F., Als-Nielsen, J., Colman, D. R., and Hendrickson, W. A. (1995) *Nature* **374**, 327–337

36. Chappuis-Flament, S., Wong, E., Hicks, L. D., Kay, C. M., and Gumbiner, B. M. (2001) *J. Cell Biol.* **154**, 231–243

37. Riehl, R., Johnson, K., Bradley, B., Grunwald, G. B., Cornel, E., Lilienbaum, A., and Holt, C. E. (1996) *Neuron* **17**, 837–848

38. Fannon, A. M., and Colman, D. R. (1996) *Neuron* **17**, 423–434

39. Inoue, A., and Sanes, J. R. (1997) *Science* **276**, 1428–1431

40. Walsh, F. S., and Doherty, P. (1997) *Annu. Rev. Cell Dev. Biol.* **13**, 425–456

41. Blaschuk, O. W., Sullivan, R., David, S., and Pouliot, Y. (1999) *Dev. Biol.* **219**, 227–229

42. Sivasankar, S., Gumbiner, B., and Leckband, D. (2001) *Biophys. J.* **80**, 1758–1768

43. Williams, E.-J., Furness, J., Walsh, F. S., and Doherty, P. (1994) *Development* **120**, 1685–1693

44. Williams, E.-J., Mittal, B., Walsh, F. S., and Doherty, P. (1995) *J. Cell Sci.* **108**, 3523–3530

45. Kim, J. B., Islam, S., Kim, Y. J., Prudoff, R. S., Saus, K. M., Wheelock, M. J., and Johnson, K. R. (2000) *J. Cell Biol.* **151**, 1193–1206
