Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6022–6026, June 1995
Neurobiology

Contactus adherens, a special type of plaque-bearing adhering junction containing M-cadherin, in the granule cell layer of the cerebellar glomerulus

(cytoskeleton/cell adhesion molecules/desmosomes/cadherins/neurons)

OLAF ROSE*, CHRISTINE GRUND†, SIGRID REINHARDT‡, ANNA STARZINSKI-POWITZ*, AND WERNER W. FRANKE†§

*Department of Anthropology and Human Genetics, University of Frankfurt, D-60054 Frankfurt, Federal Republic of Germany; †Division of Cell Biology, German Cancer Research Center, D-69009 Heidelberg, Federal Republic of Germany; and ‡Department of Physiological Chemistry, University of Mainz, D-55099 Mainz, Federal Republic of Germany

Communicated by François Jacob, Institut Pasteur, Paris, France, March 22, 1995 (received for review October 7, 1994)

ABSTRACT **In the glomeruli of the granule cell layer of mammalian cerebellum, neuronal extensions are interconnected by numerous small, nearly isodiametric (diameters up to 0.1 μm), junctions previously classified as puncta adherentia related to the vinculin-containing, actin microfilament-anchoring junctions of the zonula adherens of epithelial and certain other cells. Using immunofluorescence and immunoelectron microscopy, we have found, however, that these junctions are negative for E- and VE-cadherin, for desmosomal cadherins, and also for vinculin, α-actinin, and desmoplakin, but they do contain, in addition to the protein plakoglobin common to all forms of adhering junctions, the plaque proteins α- and β-catenin and the transmembrane glycoprotein M-cadherin previously found as a spread—i.e., not junction bound—plasma membrane protein in certain fetal and regenerating muscle cells and in satellite cells of adult skeletal muscle. We conclude that these M-cadherin-containing junctions of the granule cell layer represent a special type of adhering junction, for which we propose the term *contactus adherens* (from the Latin *contactus*, for touch, site of bordering upon, also influence), and we discuss the differences between the various adhering junctions on the basis of their molecular constituents.**

Semistable intercellular junction structures contribute to the establishment and maintenance of tissue organization on the one hand and to intracellular architecture and processes on the other. Of special importance in this respect are the "adhering junctions" (1), which appear as closely apposed plasma membrane domains reinforced by a dense cytoplasmic plaque at which filament bundles of the cytoskeleton often, but not necessarily, attach.

The dual bipolar architectural function of these adhering junctions is also manifested by their major transmembrane constituents—i.e., glycoproteins of the cadherin family of cell adhesion molecules whose extracellularly projecting domains are involved in the Ca^{2+}-mediated homophilic cell–cell coupling and whose intracellular portion determines the composition of the plaque and the specific type of filaments anchored. The various morphological forms of plaque-bearing adhering junctions have been found to share only one major constituent, the common plaque protein plakoglobin (2–5), and are grouped, on morphological and biochemical criteria, into two major categories (see also refs. 1 and 6–12):

(*i*) The variously sized and shaped actin microfilament (MF)-anchoring "adherens junctions" (zonula adherens, fascia adherens, and punctum adherens) in which "classical" (E-, N-) cadherins are associated with a specific set of plaque proteins comprising, e.g., α- and β-catenin, vinculin, α-actinin, and radixin (for reviews see refs. 13–18).

(*ii*) The intermediate-sized filament (IF)-anchoring desmosomes (maculae adherentes) whose cell type-specific isoforms of desmosomal cadherins (desmogleins Dsg1, -2, and -3 and desmocollins Dsc1, -2, and -3) are associated with, besides plakoglobin, desmoplakin I and other apparently cell type-specific proteins such as desmoplakin II, the basic protein plakophilin ("band 6 protein"), the "08L antigen," and protein "IFAP 300" (e.g., see refs. 7, 8, 10, 14, and 19–26).

With an increasing number of molecular reagents useful for the identification and subtyping of adhering junctions we have recognized that this classification into only two basic forms is an oversimplification and that other, cell type-specific, kinds of adhering junctions exist. Here, we report the identification of a distinct small plaque-bearing junction connecting neuronal extensions in the glomeruli of the granule cell layer of the cerebellum. In these "complex synapse" terminals, mossy fibers originating from neurons of different areas of the pons, the medulla oblongata, the spinal cord, and γ-aminobutyrater-gic "Golgi cells" are interconnected to several granular cells. This type of junction differs in its composition essentially from the other adhering junctions and does not fall into either of the two established categories.

MATERIALS AND METHODS

Tissues. Various human, bovine, porcine, and murine tissues, including cerebellum, were freshly obtained and snap-frozen as described elsewhere (27–29) or fixed and processed for electron microscopy (see below).

Antibodies. Rabbit antibodies against segments of the first extracellular domains, EC1–EC3, of M-cadherin have been described (30, 31). M-Cadherin antibodies commercially available (Santa Cruz Biotechnology) were also used in some experiments. Monoclonal and polyclonal antibodies against E-, VE- ("cadherin 5"), or N-cadherin (32–34), desmoplakin, desmoglein(s), desmocollin(s), plakoglobin, vinculin, and α-actinin have also been described (29), as have the antibodies against vimentin, various cytokeratins, glial filament protein, neurofilament protein NF-L, and synaptophysin (same references and ref. 35). Rabbit antibodies against murine α- and β-catenins (36, 37) were kind gifts from Stefan Butz and Rolf Kemler (Max Planck Institute for Immunobiology, Freiburg, Germany), those specific for either β- or γ- nonmuscle actin were generously provided by G. Gabbiani (Department of Pathology, University of Geneva, Switzerland), and those to members of the spectrin and protein 4.1 families of proteins were from various laboratories.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MF, microfilament; IF, intermediate-sized filament.
§To whom reprint requests should be addressed.

Immunofluorescence Microscopy. Cryostat sections of snap-frozen cerebellar tissue were fixed with acetone for 10 min at −20°C and used directly or were, after this fixation, permeabilized for 10 min with 0.1% saponin in phosphate-buffered saline (PBS; 136.8 mM NaCl/2.7 mM KCl/7.0 mM Na_2HPO_4/1.5 mM KH_2PO_4, pH 7.2), or they were fixed with 2% (wt/vol) formaldehyde (freshly made from paraformaldehyde) for 5 min, washed three times for 2 min each with PBS containing 50 mM NH_4Cl, and treated with 0.5% Triton X-100 for 5 min prior to incubation with antibodies (38).

Electron Microscopy. For conventional thin-section studies, cerebella from NMR1-mice, embryonic day 15 to postnatal day 5, were fixed with 2.5% (vol/vol) glutaraldehyde in 50 mM sodium cacodylate buffer (pH 7.2) containing 50 mM KCl and 2.5 mM $MgCl_2$ for 30 min, postfixed with 2% OsO_4 in 50 mM cacodylate buffer for 2 hr, block-stained with 0.5% uranyl acetate in water overnight, dehydrated, and embedded in Epon.

For immunoelectron microscopy, cerebella were snap-frozen in isopentane that had been cooled in liquid nitrogen. Sections of 5 μm were prepared with a Jung CM300 cryostat (Leica, Bensheim, F.R.G.), mounted on coverslips, and fixed with 2% formaldehyde in PBS for 15 min, rinsed three times for 5 min each with PBS containing 50 mM NH_4Cl, and then treated with either (*i*) 0.1% saponin in PBS for 10–15 min or (*ii*) 0.5% Triton X-100 (same buffer) for 5–10 min, and briefly rinsed with PBS.

Rabbit antibodies specific for M-cadherin were applied for 1 hr, followed by three washes with PBS, each for 5 min. As secondary antibodies, we used anti-rabbit IgG conjugated with 5-nm colloidal gold particles (Amersham Buchler, Braunschweig, F.R.G.) or anti-rabbit IgG coupled to Nanogold (Bio Trend, Cologne, F.R.G.). Specimens treated with 5-nm gold conjugate were incubated overnight, and the Nanogold samples were incubated for 4 hr. After treatment with the appropriate secondary antibodies, specimens were rinsed for 15 min with PBS. Sections were then fixed with 2.5% glutaraldehyde, washed and treated with 2% OsO_4 for 30 min as described above, and washed with double-distilled water.

Sections treated with Nanogold were further processed for silver enhancement by using the silver-enhancement kit HQ-Silver (Bio Trend) for 3 min at room temperature in the dark. Thereafter, specimens were washed with distilled water, dehydrated, and flat-embedded as described (39), and ultrathin sections were prepared with a Reichert Jung Ultramicrotome Ultracut (Leica). Electron micrographs were taken with a Siemens EM 101 and a Zeiss electron microscope EM 910.

RESULTS

Ultrastructural Organization. The numerous plaque-bearing junctions of the cerebellar glomeruli are rather small (80- to 150-nm diameter), apparently near-isodiametric, and associated with a dense plaque 20–50 nm thick (Fig. 1). At high resolution, the intermembranous material between the two lipid bilayers often reveals periodically arranged, cross-bridging threads approximately 10 nm in diameter (e.g., Fig. 1 *c* and *d*). At many junctions relatively sparse filamentous threads 4–7 nm in diameter project into the cytoplasm (e.g., arrowheads in Fig. 1 *b* and *d*), which may represent MFs, but we have never seen bundles of densely packed actin MFs or of IFs in direct attachment to these plaques. Clearly, these junctions are identical to those described by previous authors as *puncta adherentia*—i.e., as a small-sized subtype of the actin MF-anchoring, vinculin-containing group of adhering junctions closely related to the zonula adherens and fascia adherens (e.g., refs. 6, 11, 12, and 40). Therefore, it was surprising to find that the composition of these complex junctions is different from that of all so-far-known adhering junctions.

Immunofluorescence Microscopy. The cerebellum is one of the few tissues of adult mice in which M-cadherin is detected as a M_r 130,000 polypeptide by immunoblotting of electrophoretically separated proteins (Fig. 2). Immunohistochemical studies of diverse tissues of adult mice showed that, in addition to prenatal myogenic cells, satellite cells of adult skeletal muscle, and cells activated during adult muscle regeneration (27, 30, 31, 41, 42), only one further tissue was consistently labeled—i.e., the glomeruli in the granular layer of the cerebellum. Here the M-cadherin was located at distinct sites along

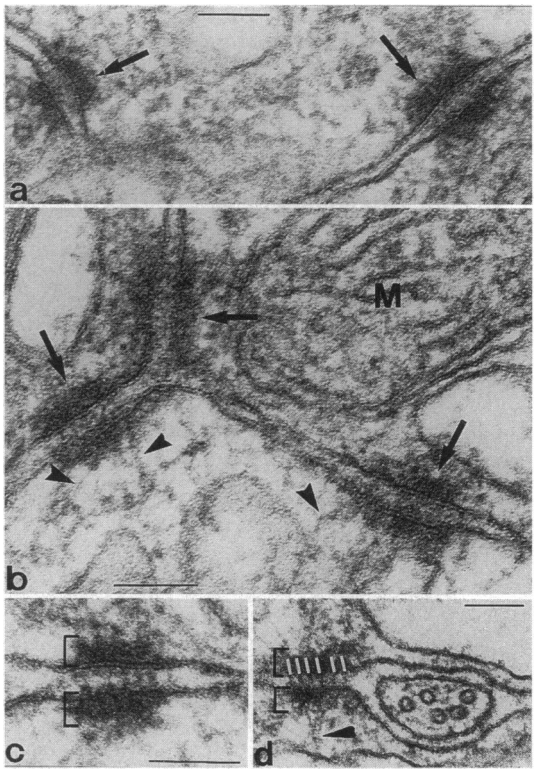

FIG. 1. Electron micrographs of ultrathin sections through the mouse cerebellum, showing various aspects of the small, plaque-bearing junctions (contactus adherentes) interconnecting several neuronal extensions in a glomerulus. (*a*) Contactus adherentes (arrows) vary somewhat in diameter, ranging from 80 nm (left) to approximately 150 nm (right), but are characterized by a relatively thick (up to 50 nm) and electron-dense plaque. (*b*) Corners of three adjacent neurites, connected by contactus adherens junctions (arrows) from which relatively sparse, nonbundled filamentous tufts project into the cytoplasm (some are denoted by arrowheads). M, mitochondrion. (*c* and *d*) Near-perpendicular (*c*) and oblique (*d*) sections revealing the regular arrays of intermembranous bridging thread structures of ≈10-nm diameter (brackets in *c*, white bars in *d*). Plaques are demarcated by bars and filamentous material projecting from the plaque by arrowhead in *d*. (Bars denote 100 nm.)

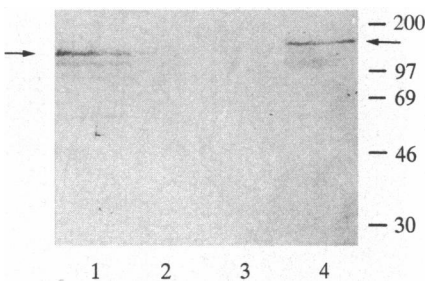

FIG. 2. Immunoblot detection of M-cadherin among proteins from murine cerebellum (lane 1), brain cortex (lane 2), liver (lane 3), and L6 myotubes isolated at day 3 after induction of myotube formation in culture (lane 4). Only the reaction with M-cadherin antibodies is shown (for protocol and details see ref. 31). The sizes of reference proteins are indicated in kDa.

nerve cell fibers of the granular cell layer (Fig. 3*a*), and at higher resolution it appeared as dense-packed clusters of small punctate structures. The M-cadherin-containing cells were identified as neuronal by their specific reaction with antibodies to synaptophysin, the major transmembrane protein of presynaptic vesicles (Fig. 3 *b* and *c*), and to the neurofilament polypeptides NF-L, NF-M, and NF-H (see also ref. 43), whereas these structures were negative for glial filament protein, desmin, and diverse cytokeratins. They were positive, however, for the common plaque protein, plakoglobin (Fig.

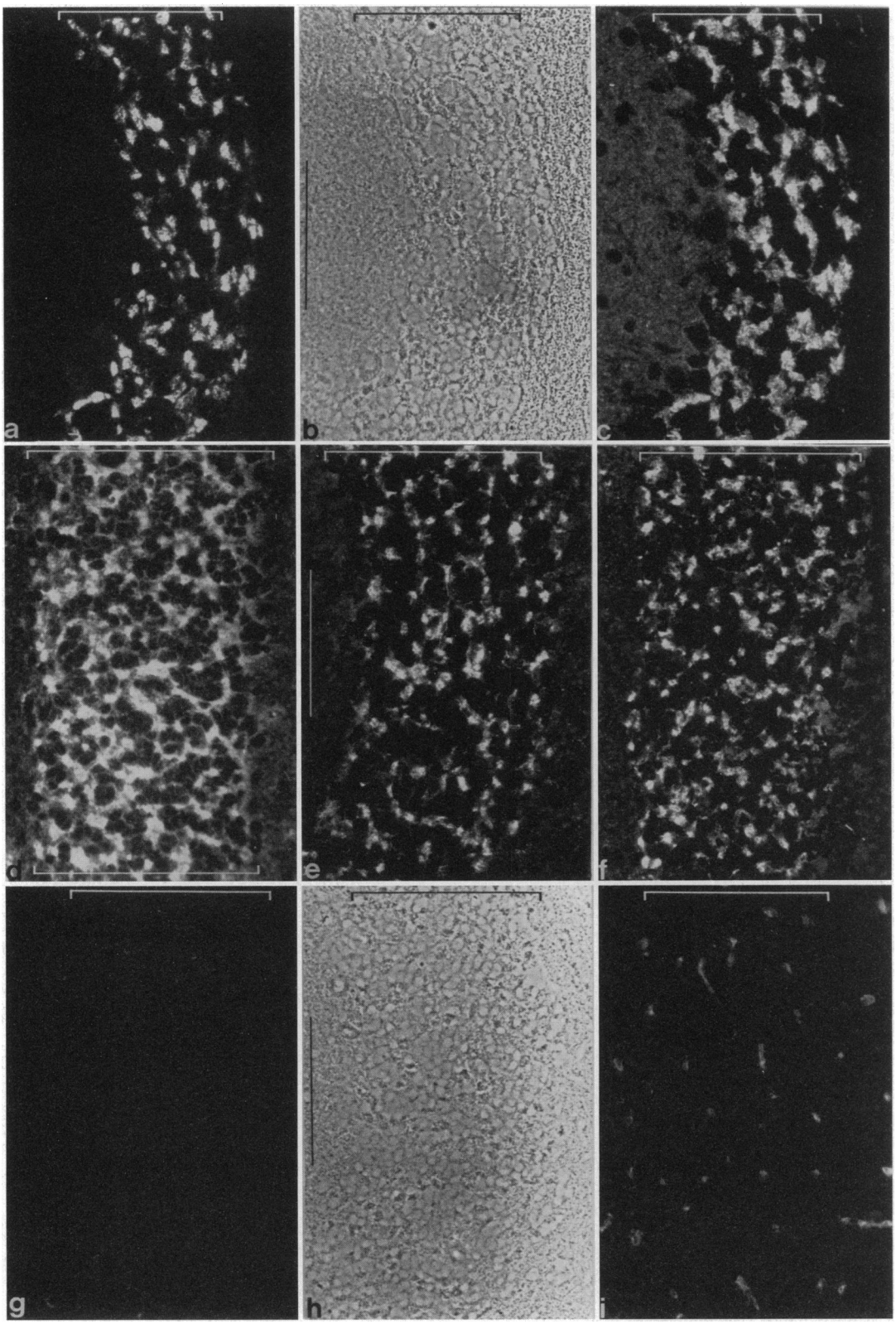

FIG. 3. Immunofluorescence micrographs of frozen sections through murine cerebellum, showing reactions of granule cell portions with antibodies to M-cadherin (*a*) in double-label comparison with antibodies to synaptophysin (*c*; *b*, same field in phase-contrast optics), plakoglobin (*d*), β-catenin (*e*), α-catenin (*f*), and desmoplakin (*g*; *h*, same field in phase-contrast optics), and vinculin (*i*). The layer of granule cells is demarcated by brackets. (Bars represent 100 μm.)

3*d*), as well as for α- and β-catenin (Fig. 3 *e* and *f*), but not for desmoplakin (Fig. 3 *g* and *h*), vinculin (Fig. 3*i*), α-actinin, desmocollins, E-cadherin, and VE-cadherin (not shown). A weak reaction, however, was seen with some N-cadherin antibodies (cf. ref. 44), whereas a faint reaction sometimes seen with antibodies to desmogleins Dsg1, -2, and -3 was regarded as not significantly above a disturbing background labeling. Antibodies to proteins of the spectrin family and to protein 4.1 and other members of the ERM family did not show significant reaction at the M-cadherin-containing junctions, in agreement with results of previous authors (e.g., refs. 45–47).

Immunoelectron Microscopy. To identify the structures labeled by M-cadherin antibodies we used various protocols of immunoelectron microscopy, including pre-embedding immunogold labeling on frozen sections with or without saponin treatment. These studies (examples using the silver enhancement technique are presented in Fig. 4) revealed the plaque-bearing small junctions shown in Fig. 1 as the only significantly labeled structure. At higher magnifications, cross sections perpendicular to the symmetry plane of these junctions showed the immunolabel exclusively localized to the central "space" between the lipid bilayer structures of the membrane domains—i.e., the region in which the extracellular portions of cadherins of one neurite interact with those of the neighboring neurite (Fig. 4 *c–f*). Thus, this localization, the intensity of which was enhanced after pretreatment with the biological detergent saponin, corresponded to the location of the epitopes, as these antibodies had been directed against peptide sequences of the extracellular domains EC1–EC3 (31). We never found M-cadherin immunoreactivity located in the area of synapses (Fig. 4*a*). The submembranous plaques of any other plasma membrane structures of the brain were negative.

Confirming our light microscopic observations, the plaques of the cerebellar junctions were positive for M-cadherin, plakoglobin, and catenins (not shown).

The granule cell structures containing the M-cadherin-positive junctions were also intensely immunostained with antibodies to nonmuscle actins (Fig. 5*a*). Immunoelectron microscopy, however, revealed that most of this immunogold-labeled actin was associated with synaptic densities (Fig. 5*b*), whereas the junctional plaques appeared mostly free of actin label (Fig. 5 *b–e*). In contrast, occasional arrays of individual or loosely fasciated filaments approaching the plaques of these junctions were often decorated with actin antibodies (Fig. 5 *c–e*).

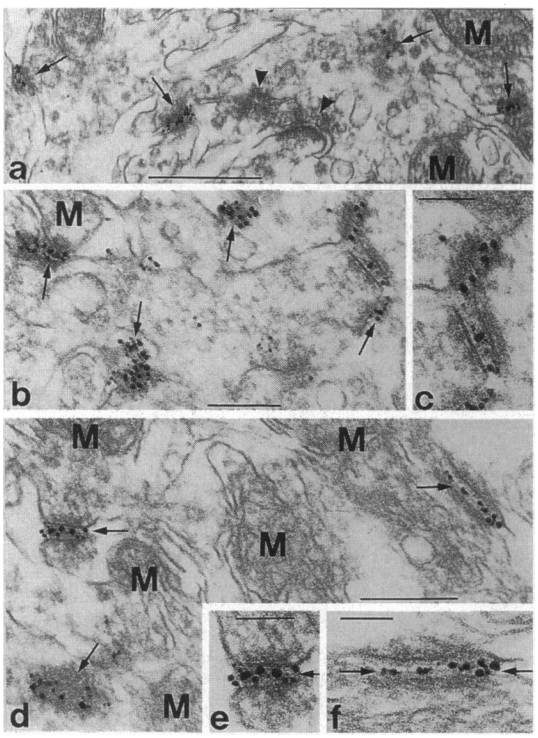

FIG. 4. Immunoelectron microscopy of unfixed frozen sections through cerebellar glomeruli of mouse brain, showing several neurites after pre-embedding immunogold labeling, using M-cadherin antibodies, and subsequent enhancement of the Nanogold particles by the "silver enhancement" technique. (*a*) Survey micrograph showing the specificity of the immunogold labeling on the contactus adherens junctions connecting neurites (arrows), whereas synapses (arrowheads), mitochondria (M), and all other structures are not significantly labeled. (*b*) Details of the immunolabeling of contactus adherens junctions in grazing (the two arrows in the left), oblique (arrow in the middle), and cross (the arrow in the right) sections. (*c*) Higher magnification of the right part of *b*, showing, in cross sections through three individual adjacent junctions, that the immunolabel particles are concentrated over the space between the lipid bilayer structures of the junctional membranes. (*d*) Survey micrograph showing M-cadherin labeling of the contactus adherens junctions (arrows) between mitochondria (M)-rich portions as seen in grazing (lower arrow) and cross (upper arrow) sections. (*e* and *f*) Higher magnification of the junctions denoted by the two upper arrows in *d*, showing the specific restriction of immunolabel gold and silver particles (arrows) to the material located in the intermembranous portion sandwiched between the two lipid bilayers, reflecting the fact that these antibodies have been raised against—and are specific for—the extracellular domain of M-cadherin. [Bars represent 500 nm (*a*), 250 nm (*b* and *d*), and 100 nm (*c*, *e*, and *f*).]

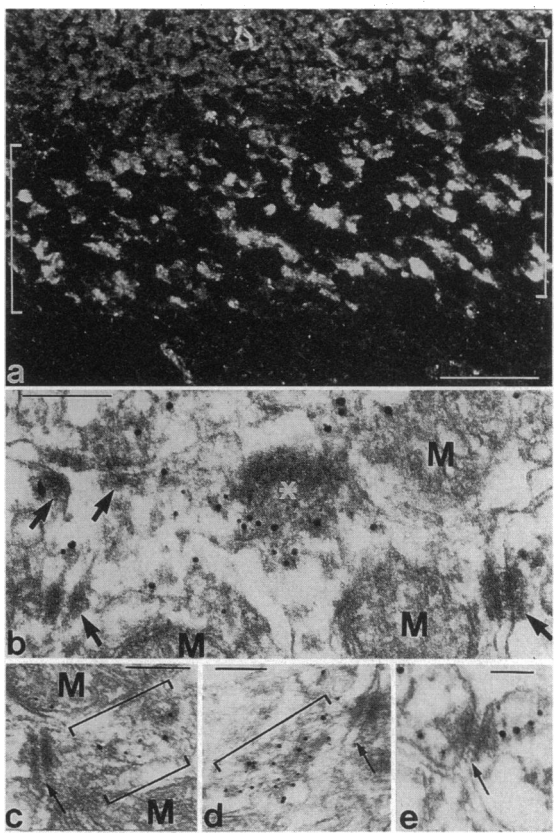

FIG. 5. Reaction of cells of the granular cell layer of murine cerebellum (demarcated by bracket in *a*) with antibodies to nonmuscle β-actin, visualized by immunofluorescence microscopy (*a*) or by electron microscopy using immunogold label (*b* and *c*) on sections from the same block of frozen tissue. Note absence of significant reaction on the contactus adherens plaques proper (some are denoted by arrows), in contrast to the immunogold particle decoration of MF arrays approaching such plaques (denoted by brackets in *c* and *d*; an individual thin MF bundle is seen in *e*) and the synaptic densities [one is seen in a grazing section in *b* (white asterisk)]. M, mitochondria. [Bars represent 50 μm (*a*), 0.2 μm (*b–d*), and 0.1 μm (*e*).]

DISCUSSION

The numerous small, plaque-bearing junctions in the cerebellar glomeruli which interconnect mossy fibers and terminals of Golgi cells with granular cells have so far been classified as typical puncta adherentia, literally as "miniature versions" of the zonula adherens and related junctions containing E-cadherin and a plaque rich in plakoglobin and vinculin, which anchors actin IFs (ref. 40; for reviews see also refs. 6 and 12). Our present results demonstrate that the small granule cell junctions discussed here contain plakoglobin—like all other known adhering junctions—but otherwise differ markedly in composition, as they do not contain vinculin and E- or VE-cadherin but instead are characterized by a different cadherin, M-cadherin, so far known as a general laterally spread—i.e., not junction-enriched—plasma membrane component exclusive to myoblasts and satellite cells of fetal and regenerating skeletal muscle (27, 30, 31, 41, 43). We conclude that these M-cadherin-containing junctions represent a type of adhering junction *sui generis*, for which we propose the term *contactus adherens* (from the Latin word *contactus*, for touch, site of bordering upon, also influence). Whether this type of junction contains further specific, yet-unknown, transmembrane or plaque constituents remains to be examined.

In the adult mouse, M-cadherin immunoreactivity was not detectable in other parts of the brain, with the possible exception of ependymal cells, or in various peripheral nerves, and it was also absent from diverse nonneural and nonmuscle tissues (refs. 27 and 31; unpublished observations). This indicates that the small similar-looking, plaque-bearing junctions that occur in a variety of different cell types and so far have been collectively referred to as puncta adherentia are in fact a compositionally—and probably also functionally—heterogenous group. Obviously, the term *puncta adherentia*, following the original definition as a morphological variant of the zonula adherens, should be reserved for those small adhering junctions that are indeed compositionally similar to MF-anchoring, zonula adherens-related junctions. Clearly, for distinguishing and classifying the different punctum-like, plakoglobin-positive junctions, the determination of the specific type of cadherin present will be of particular importance. Recently, we have shown that another form of plaque-bearing adhering junction, the extensive zonula-like structures connecting endothelial cells of various kinds of lymphatic vessels, differs profoundly from desmosomes and the zonula adherens by the absence of desmosomal cadherins and E-cadherin as well as of vinculin and α-actinin and by the combination of the plaque proteins plakoglobin and desmoplakin with the endothelium-specific VE-cadherin. These endothelial cell junctions therefore represent another distinct kind of adhering junction, termed the *complexus adherens* (29, 38).

As we have shown that the small plaque-bearing junctions between neurites in the cerebellar glomeruli represent a distinct type of junction containing M-cadherin, the question arises what the specific functions of the contactus adherens and its M-cadherin in this brain tissue might be, both during granule cell migration in the developing cerebellar cortex (for review see ref. 48) and later in the mature tissue. So far, the neuronal extensions in the glomeruli could not be assigned to one particular neuronal cell type. The extensions might belong to neurons which are located in the pons, the medulla oblongata, or the spinal cord, or in granular cells of the cerebellum itself. The functional importance of the granule cells of this region is indicated by the weaver mutant mouse, in which most of these cells die during development (49–52).

We thank Ms. Irmgard Weirich for typing the manuscript, Ms. Sibille Engels for preparing the cerebella, and the Deutsche Forschungsgemeinschaft for supportive grants (to A.S.-P., S.R., and W.W.F.).

1. Farquhar, M. & Palade, G. E. (1963) *J. Cell Biol.* **17,** 375–412.
2. Cowin, P., Kapprell, H.-P., Franke, W. W., Tamkun, J. & Hynes, R. O. (1986) *Cell* **46,** 1063–1073.
3. Franke, W. W., Kapprell, H.-P. & Cowin, P. (1987) *Eur. J. Cell Biol.* **43,** 301–315.
4. Franke, W. W., Kapprell, H.-P. & Cowin, P. (1987) *Biol. Cell* **49,** 205–218.
5. Franke, W. W., Goldschmidt, M. D., Zimbelmann, R., Mueller, H. M., Schiller, D. L. & Cowin, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4027–4031.
6. Fawcett, D. W. (1981) *The Cell* (Saunders, Philadelphia), 2nd Ed.
7. Franke, W. W., Schmid, E., Grund, C., Müller, H., Engelbrecht, I., Moll, R., Stadler, J. & Jarasch, E.-D. (1981) *Differentiation* **20,** 217–241.
8. Franke, W. W., Moll, R., Schiller, D. L., Schmid, E., Kartenbeck, J. & Müller, H. (1982) *Differentiation* **23,** 115–127.
9. Geiger, B., Volberg, T., Sabanay, I. & Volk, T. (1990) in *Morphoregulatory Molecules*, eds. Edelman, G. M. & Thiery, J. P. (Wiley, New York), pp. 57–79.
10. Schäfer, S., Troyanovsky, S. M., Heid, H. W., Eshkind, L. G., Koch, P. J. & Franke, W. W. (1993) *C. R. Acad. Sci. Ser. III* **316,** 1316–1323.
11. Staehelin, L. A. (1974) *Int. Rev. Cytol.* **39,** 191–283.
12. Weiss, L. (1988) *Cell and Tissue Biology* (Urban & Schwarzenberg, Baltimore).
13. Drenckhahn, D. & Franz, H. (1986) *J. Cell Biol.* **102,** 1843–1852.
14. Heid, H. W., Schmidt, A., Zimbelmann, R., Schäfer, S., Winter-Simanowski, S., Figge, U., Schnölzer, M. & Franke, W. W. (1994) *Differentiation*, in press.
15. Kemler, R. (1992) *Semin. Cell Biol.* **3,** 149–155.
16. Peifer, M., McCrea, P., Green, K. J., Wieschaus, E. & Gumbiner, B. (1992) *J. Cell Biol.* **118,** 681–691.
17. Tsukita, S., Tsukita, S., Nagafuchi, A. & Yonemura, S. (1992) *Curr. Opin. Cell Biol.* **4,** 834–839.
18. Tsukita, S., Itoh, M., Nagafuchi, A., Yonemura, S. & Tsukita, S. (1993) *J. Cell Biol.* **123,** 1049–1053.
19. Buxton, R. W. & Magee, A. I. (1992) *Semin. Cell Biol.* **3,** 157–167.
20. Buxton, R. S., Cowin, P., Franke, W. W., Garrod, D. R., Green, K. J., King, I. A., Koch, P. J., Magee, A. I., Rees, D. A., Stanley, J. R. & Steinberg, M. S. (1993) *J. Cell Biol.* **121,** 481–483.
21. Cowin, P., Kapprell, H.-P. & Franke, W. W. (1985) *J. Cell Biol.* **101,** 1442–1454.
22. Garrod, D. R. (1993) *Curr. Opin. Cell Biol.* **5,** 30–40.
23. Ouyang, P. & Sugrue, S. P. (1992) *J. Cell Biol.* **118,** 1477–1488.
24. Schäfer, S., Koch, P. J. & Franke, W. W. (1994) *Exp. Cell Res.* **211,** 391–399.
25. Schwarz, M. A., Owaribe, K., Kartenbeck, J. & Franke, W. W. (1990) *Annu. Rev. Cell Biol.* **6,** 461–491.
26. Skalli, O., Jones, J. C. R., Gagescu, R. & Goldman, R. D. (1994) *J. Cell Biol.* **125,** 159–170.
27. Irintchev, A., Zeschnigk, M., Starzinski-Powitz, A. & Wernig, A. (1994) *Dev. Dyn.* **199,** 326–337.
28. Moll, R., Löwe, A., Laufer, J. & Franke, W. W. (1992) *Am. J. Pathol.* **140,** 427–447.
29. Schmelz, M., Moll, R., Kuhn, C. & Franke, W. W. (1994) *Differentiation* **57,** 97–117.
30. Donalies, M., Cramer, M., Ringwald, M. & Starzinski-Powitz, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8024–8028.
31. Rose, O., Rohwedel, J., Reinhardt-Maelicke, S., Bachmann, M., Cramer, M., Rotter, M., Wobus, A. & Starzinski-Powitz, A. (1994) *Dev. Dyn.* **201,** 245–259.
32. Lampugnani, M. G., Resnati, M., Raiteri, M., Pigott, R., Piscane, A., Houen, G., Puco, L. P. & Dejana, E. (1992) *J. Cell Biol.* **118,** 1511–1522.
33. Suzuki, S., Sano, K. & Tanihara, H. (1991) *Cell Regul.* **2,** 261–270.
34. Alexander, J. S., Blaschuk, O. W. & Haselton, F. R. (1993) *J. Cell. Physiol.* **156,** 610–618.
35. Wiedenmann, B. & Franke, W. W. (1985) *Cell* **41,** 1017–1028.
36. Herrenknecht, K., Ozawa, M., Eckerskorn, C., Lottspeich, F., Lenter, M. & Kemler, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9156–9160.
37. Butz, S., Stappert, J., Weissig, H. & Kemler, R. (1992) *Science* **257,** 1142–1144.
38. Schmelz, M. & Franke, W. W. (1993) *Eur. J. Cell Biol.* **61,** 274–289.
39. Franke, W. W., Schmid, E., Osborn, M. & Weber, K. (1978) *Cytobiologie* **17,** 365–391.
40. Peters, A., Palay, S. L. & Webster, H. de F. (1976) *The Fine Structure of the Nervous System: The Neurons and Supporting Cells* (Saunders, Philadelphia).
41. Bornemann, A. & Schmalbruch, H. (1994) *Anat. Rec.* **239,** 119–125.
42. Moore, R. & Walsh, F. S. (1993) *Development (Cambridge, U.K.)* **117,** 1409–1420.
43. Matus, A. I., Meelian, N. G. & Jones, D. H. (1979) *J. Neurocytol.* **8,** 513–525.
44. Redies, C. & Takeichi, M. (1993) *Dev. Dyn.* **197,** 26–39.
45. Sato, N., Funayama, N., Nagafuchi, A., Yonemura, S., Tsukita, S. & Tsukita, S. (1992) *J. Cell Sci.* **103,** 131–143.
46. Franck, Z., Gary, R. & Bretscher, A. (1993) *J. Cell Sci.* **105,** 219–231.
47. Berryman, M., Franck, Z. & Bretscher, A. (1993) *J. Cell Sci.* **105,** 1025–1043.
48. Rakic, P. (1985) in *Molecular Mechanisms of Neural Development*, eds. Edelman, G. M., Gall, W. E. & Cowan, W. M. (Wiley, New York), pp. 139–160.
49. Pickford, L. B., Mayer, D. N., Bolin, L. M. & Rouse, R. V. (1989) *J. Neurocytol.* **18,** 465–478.
50. Rakic, P. & Sidman, R. L. (1973) *J. Comp. Neurol.* **152,** 103–132.
51. Rakic, P. & Sidman, R. L. (1973) *J. Comp. Neurol.* **152,** 133–162.
52. Rakic, P. & Sidman, R. L. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 240–244.