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Original Article

Expression of the Neural Cell Adhesion Molecule NCAM in Endocrine Cells[1]

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We examined the expression of the neural cell adhesion molecule NCAM in a number of endocrine tissues of adult rat and in an endocrine tumor cell line. NCAM was found by immunoelectron microscopy to be present on the surface of all endocrine cells in the three lobes of the hypophysis, although staining was relatively less intense in the intermediate lobe, and in pancreatic islets. Pituicytes, hypophyseal glial cells, were also labeled for NCAM. A rat insulinoma cell line (RIN A2) also expressed NCAM as judged by immunocytochemistry. Analysis of NCAM antigenic determinants (M_r 180, 140, and 120 KD) revealed large variations in the relative proportions of NCAM polypeptides present in the different tissues. Although all tissues and cell lines expressed NCAM-140, NCAM-180 was not detected in the adenohypophysis, pancreas, or adrenal medulla, and NCAM-120 was found in none of the endocrine tissues or cell lines except at low levels in the neurohypophysis. The tumor cell line expressed significant levels of NCAM-180, which was most abundant in the neurohypophysis. These results show that NCAM expression appears to be a general property of endocrine cells, although the antigenic composition differs markedly from that in brain tissue. These data are discussed with regard to the embryological origins of the different endocrine tissues, and possible functional implications are suggested. (*J Histochem Cytochem 37:781-791, 1989*)

KEY WORDS: Neural cell adhesion molecule NCAM; Endocrine cells; Immunocytochemistry; Immunoblots.

Introduction

Intercellular adhesion has long been considered to provide a possible basis for tissue formation (see Moscona, 1976, for review). More recently, it has been suggested that differential expression of cell adhesion molecules (CAM), on either a temporal or a topographical basis, is of fundamental importance to neuro-ontogenesis (for reviews, see Edelman, 1983,1984; Rutishauser, 1984; Goridis et al., 1983). Several distinct CAM have been discovered; the most well known of them, studied independently under the names of NCAM (Thiery et al., 1977), D-2 protein (Jorgensen and Bock, 1974), and BSP-2 (brain surface protein-2) (Hirn et al., 1981) is considered to mediate cell adhesion by a Ca^{2+}-independent homophilic binding mechanism (Hoffman and Edelman, 1983). NCAM exists in adult brain as a family of sialoglycoproteins of M_r 180, 140, and 120 KD, the relative proportions of which vary slightly from one region to another (Chuong and Edelman, 1984). The differences in molecular weights of these NCAM determinants are essentially due to differences in the lengths of their C-terminal domains (Cunningham et al., 1983,1987; Gennarini et al., 1984), which for the two larger polypeptides are cytoplasmic.

Variations in cell surface density of NCAM or in its chemical constitution modulate cell adhesivity (Edelman, 1984; Goridis et al., 1983). Therefore, it is essential to elucidate both cellular expression of individual CAM and their biochemical nature to fully understand the molecular mechanisms that lead to formation of a given tissue. Different primary CAM have been broadly classified according to the type of cell that expresses them in adult tissues. Immunocytochemical data indicate that in the adult NCAM is expressed by a wide variety of (probably all) neurons (Langley et al., 1983; Rutishauser et al., 1978a, b), certain glial cells including astrocytes and Schwann cells (Covault and Sanes, 1986; Van den Pol et al., 1986; Langley and Aunis, 1984; Langley et al., 1982), and by certain neuroendocrine cells, so-called paraneurons, including chromaffin cells of the adrenal medulla and tumor lines derived from them (Langley and Aunis, 1984; Jorgensen and Richter-Landsberg, 1983). In early embryos, however, cells of all three primitive layers have been shown to express NCAM (Crossin et al., 1985), and at later embryonic developmental stages NCAM has been found in several non-neural tissues (Murray et al., 1986). In contrast, NCAM expression in adult tissues has been considered to be mainly

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limited to cells of the nervous system. Apart from the cell types listed above, all of which are of neural tube or neural crest origin, adult skeletal muscle has been shown to display limited expression of NCAM at the neuromuscular junction, although embryonic or regenerating injured muscle cells express the antigen over their entire surface (Covault and Sanes, 1986; Sanes et al., 1986). Indications that NCAM may be expressed by other non-neural cells in the adult have been suggested by a report that expression of the molecule in chick lung epithelium persists in the adult (Crossin et al., 1985), and by data concerning feather regeneration in adult chickens (Chuong and Edelman, 1984). A recent report also suggests that NCAM is present in adult intestine (Thor et al., 1987).

In the present study, our previous investigations of endocrine cells of neural origin (Langley et al., 1987; Langley and Aunis, 1984) were extended to cover endocrine tissues of both neural and non-neural origin and a rat insulinoma cell line. Immunocytochemistry was used to provide unambiguous data on cellular distribution of the antigens; in addition, the nature of the NCAM polypeptides present in the tissues was investigated immunochemically. The present data suggest that NCAM expression may be a general feature of endocrine cells, whatever the nature of their embryonic origin. In addition, the pattern of the NCAM forms in the various endocrine cells studied was found to differ markedly from that found in the central nervous system.

Materials and Methods

Tissue Preparation. Adult male or female rats (150–200 g) were killed under sodium pentobarbital anesthesia by transcardiac perfusion for 30 min with freshly prepared formaldehyde (4% in 0.12 M phosphate buffer, pH 7.2). The hypophysis and pancreas were removed and post-fixed in the same fixative for 2 hr. Vibratome sections were cut 50–100 μm thick. To facilitate sectioning of the pancreas, small blocks of tissue were first mounted in 3% agarose. The hypophysis was sectioned starting at the hypophyseal stalk.

For immunochemistry, the cerebellum and the posterior lobe of the hypophysis were dissected from adult male or female rats. The tissue was cut into small pieces, placed in extraction medium (62.5 mM Tris, 2% SDS, 2 mM EDTA-Na_2, 2 mM PMSF, 15% sucrose, pH 6.8) and homogenized in a teflon-to-glass homogenizer. Pancreatic islets were isolated by microdissection under a binocular microscope from pancreatic tissue stained with neutral red as previously described (Winckler, 1974). They were homogenized immediately in extraction medium by sonication. All homogenates were boiled for 5 min and centrifuged at 13,000 × g for 4 min. The protein concentrations of the supernatants were determined by the bicinchoninic acid protein assay (Smith et al., 1985).

Tissue Cultures. The rat insulinoma cell line (RIN A2; kindly provided by H.P.T. Ammon, Dept. of Pharmacology, University of Tübingen, FRG) was cultivated in RPMI 1640 (supplemented with mycoplasma-free 10% heat-inactivated fetal calf serum) on plastic tissue culture dishes as previously described (Lind et al., 1987).

For immunocytochemistry, cells were fixed in freshly prepared phosphate-buffered formaldehyde (0.12 M, pH 7.2; 4%) for 30 min after brief rinsing in PBS. For immunochemistry the cells were lysed with the same extraction medium used for the tissues, boiled, and centrifuged as described above.

Immunocytochemistry. Cell cultures or free-floating vibratome tissue sections were pre-incubated in normal sheep serum (NSS; 5% in PBS, 30 min) and then in polyclonal rabbit serum directed against immunoaffinity-purified NCAM (a generous gift of Dr. C. Goridis; dilution 1:1000–1:3000 in PBS containing 1% NSS, 1 hr at room temperature for cultures or overnight at 4°C for tissue sections). Cultures and tissues were then extensively washed in PBS and incubated in either fluorescein isothiocyanate- or peroxidase-conjugated sheep anti-rabbit immunoglobulins (Institut Pasteur; 2 hr, dilution 1:100 in PBS containing 1% NSS). Preparations were extensively washed with PBS and viewed either directly by epifluorescence with appropriate filters or after development of tissue-bound peroxidase with diaminobenzidine and hydrogen peroxidase (0.05%; 0.01%, 4 min in the dark, followed by further washes in PBS). Immunoperoxidase-labeled preparations were observed by brightfield optics without counterstaining. Samples destined for electron microscopy were further treated with osmium tetroxide (1% in sodium cacodylate buffer in 0.1 M, pH 7.2, 1 hr) and dehydrated in graded ethanols. Cultures were embedded still attached to the coverslips by placing inverted tubes filled with Spurr's resin over them. After polymerization, coverslips were removed with liquid nitrogen. Vibratome sections were flat-embedded between strips of solvent-resistant plastic. Gold ultra-thin sections were viewed without counterstaining with a Philips EM 420 operated at 60 or 80 kV using a 20-μm objective aperture.

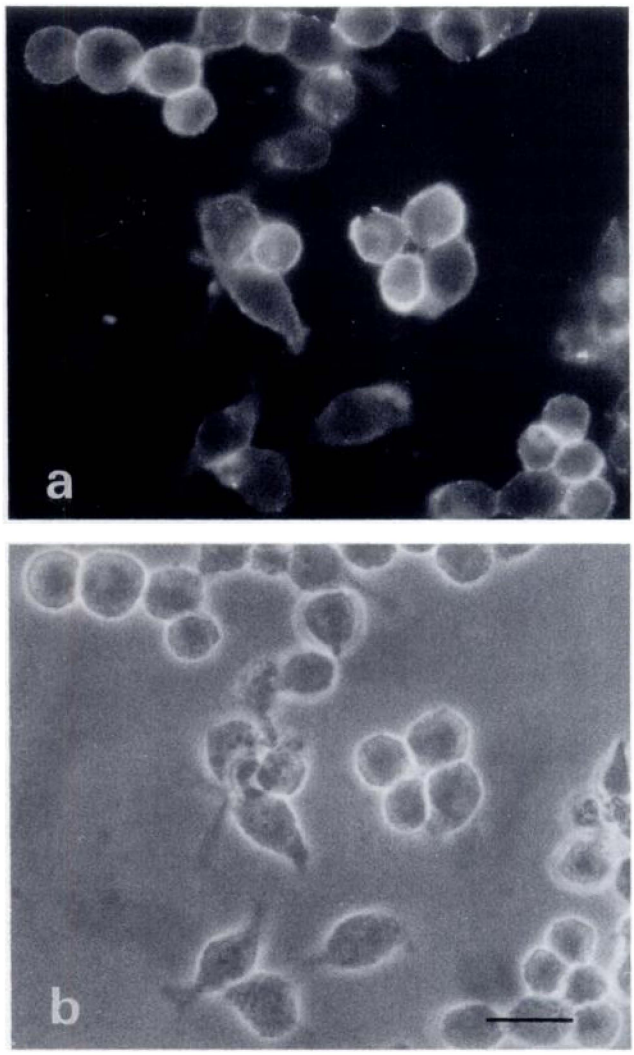
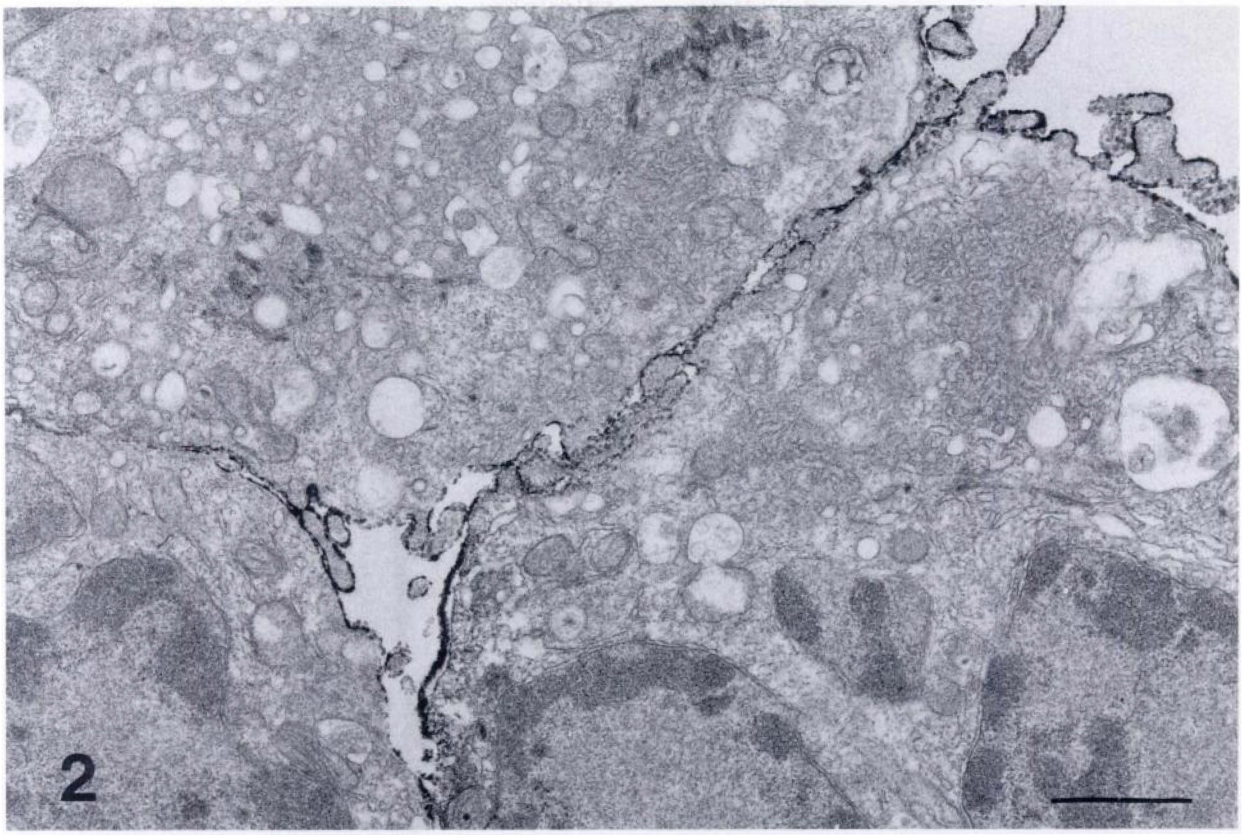


Figure 1. Rat insulinoma cell culture labeled for NCAM, photographed by epifluorescence (a) or phase-contrast (b) optics. Bar = 20 μm.



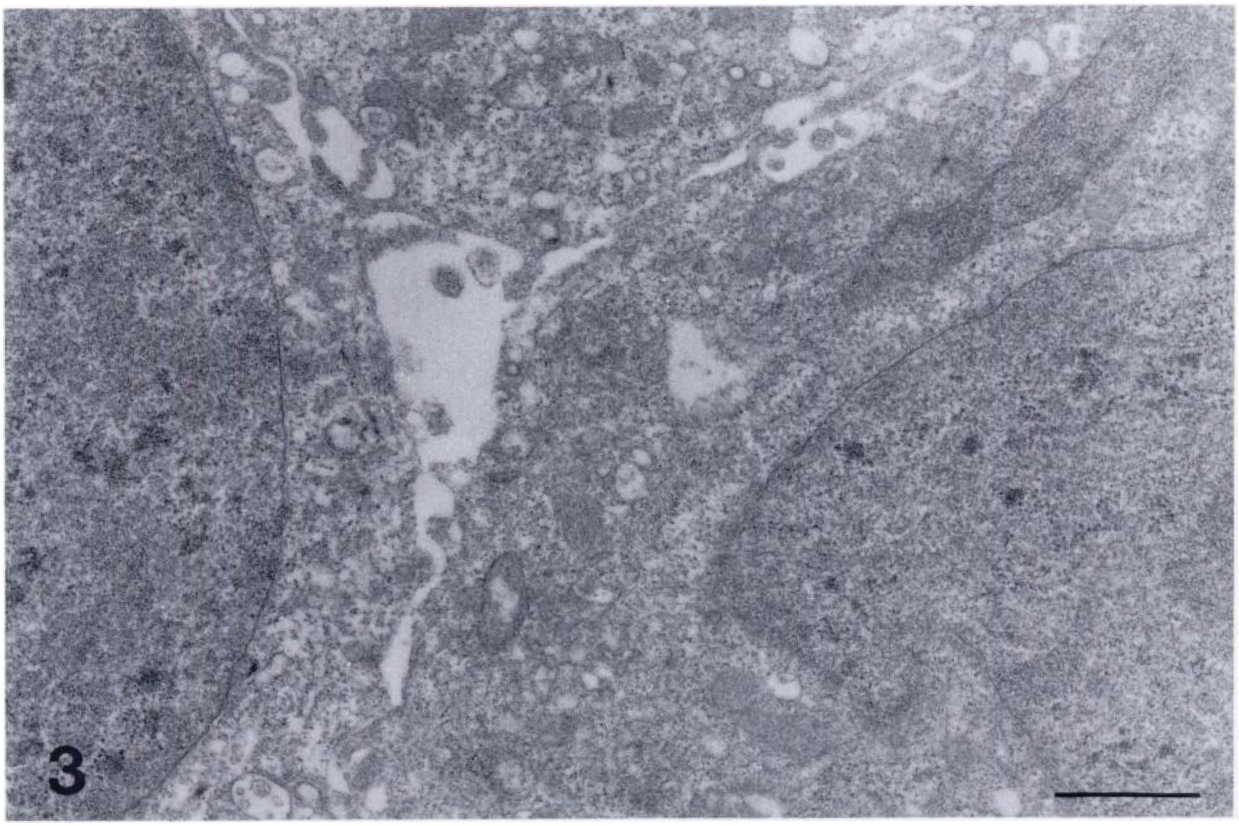


Figure 2. Electron micrograph of rat insulinoma cell culture labeled for NCAM. Immunoperoxidase reaction product is localized over the entire surface membrane but is not detectable in the cytoplasm. Section not counterstained. Bar = 1 μm.

Figure 3. Electron micrograph of control rat insulinoma cell culture immunostained with normal rabbit serum replacing anti-NCAM serum. No surface labeling is detectable. Section not counterstained. Bar = 1 μm.

Controls were run in parallel, with normal rabbit serum replacing anti-NCAM serum at the same dilution.

Immunochemistry. The proteins extracted from the tissues and cultures as described above were subjected to SDS-PAGE (7.5%) under reducing conditions (50–100 μg/lane) (Laemmli, 1970). They were transferred to nitrocellulose paper (BA 85; Schleicher and Schull, Dassel, FRG) at 60 V for 3 hr (Towbin et al., 1979). The transfer buffer contained 25 mM Tris, 192 mM glycine, and 20% methanol. To block nonspecific protein binding sites the blots were washed four times for 5 min at room temperature with TBS–Tween (150 mM NaCl, 50 mM Tris, 0.05% Tween-20, pH 7.6) containing 2.5% lipid-free instant milk, and then washed 5 min with TBS–Tween containing 0.5% instant milk. Anti-NCAM antiserum was used at a 1:1000 dilution in TBS–Tween. After incubation overnight at 4°C the blots were washed four times for 15-min periods with TBS–Tween and then incubated in ^{125}I-labeled protein A/TBS–Tween (1 μCi/ml) for 1 hr [iodinated protein A was prepared according to the method described by Greenwood and Hunter (1963)]. After washing with TBS–Tween (three times for 1 hr) the labeled material was detected by autoradiography (Kodak X Omat AR film). The nature of a 49 KD band, stained on immunoblots by the NCAM antiserum used, was investigated by blot-affinity purification (Olmsted, 1986). Antibodies bound to the proteins to be analyzed after transfer to nitrocellulose strips were eluted with ice-cold 0.2 M glycine-HCl (pH 2.8) for 5 min. The eluted material was immediately neutralized to pH 7 with disodium hydrogen phosphate, lyophilized, dissolved in a small volume of distilled water, and dialyzed against TBS–Tween.

For quantification of NCAM polypeptides, the optical densities of the immunolabeled bands were determined by computer-assisted analysis (IPS; Kontron, FRG). The relative areas of peaks were determined (MOP AMO2; Kontron) and expressed as percentages.

Results

Immunocytochemistry

Insulinoma Cells. Under the conditions employed here, the insulinoma cultures grew as clumps of round cells from which occasional cell processes extended. Cultures labeled for NCAM by immunofluorescence displayed bright surface fluorescence with dark cytoplasm (Figure 1). An identical staining pattern was obtained with the immunoperoxidase method. Cell extensions were also surface labeled. Electron microscopy revealed two principal differences in the morphology of these cells compared with their normal in vivo counterparts (pancreatic islet B-cells). Secretory granules were relatively scarce, and bundles of intermediate filaments similar to those described in PC12 cells (Franke et al., 1986) were prominent. Ultrastructural observations of immunoperoxidase-labeled cultures confirmed the presence of specific reaction product, indicating the presence of anti-NCAM reactive antigens over the entire surface of these cells (Figure 2). Omega figures and occasional small vesicles close to the surface membrane, presumably interconnected with it, were also labeled, as were cell extensions. Controls were completely negative (Figure 3).

Pancreas. The remarkable feature of pancreas labeled for NCAM, as seen by light microscopy, was the pronounced surface staining cells of the islets of Langerhans (Figure 4). In contrast, acinar cells of exocrine pancreas were apparently little or unstained. A somewhat variable but low level of staining, presumed to be extracellular, was sometimes apparent between groups of acinar cells. Electron microscopy showed that all secretory cells of pancreatic islets reacted intensely with anti-NCAM antibodies (Figure 5). Labeling was confined to the surface membrane but was occasionally found in the cytoplasm close to the surface membrane. No other significant intracellular staining was observed. Acinar cells were unstained or very weakly stained, and capillary endothelial cells were negative. Some extracellular staining was apparent, particularly in pancreatic islets. Elements of the basement membrane, and collagen fibrils in particular, were decorated with peroxidase reaction product. Although this staining was less intense than that of the islet cell surface staining, it could not be eliminated by using higher dilutions of primary antibody, which still resulted in islet cell surface labeling. Normal rabbit serum controls produced none of these specific labeling features.

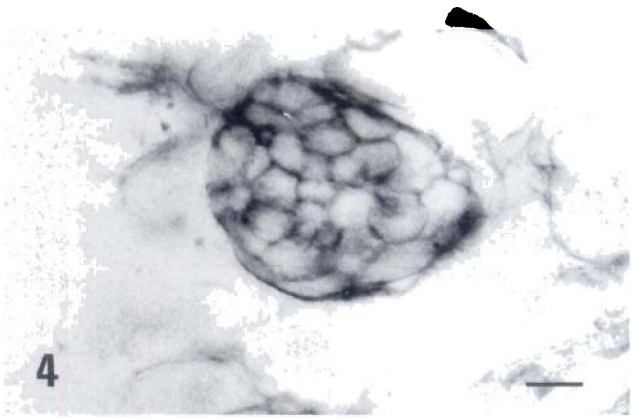
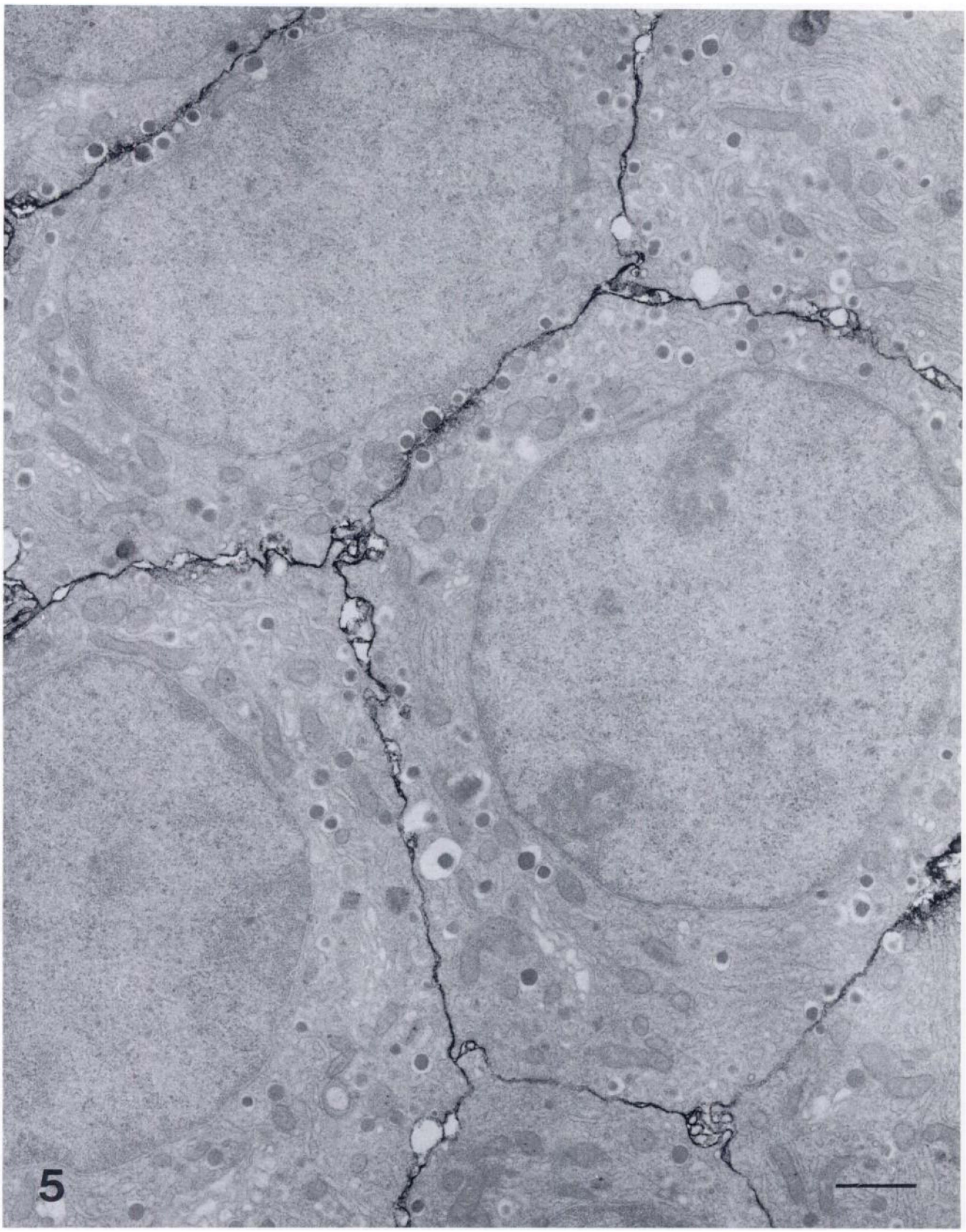


Figure 4. Vibratome section of rat pancreas labeled for NCAM. Note intense immunoperoxidase surface staining of all secretory cells of the islet of Langerhans and absent or very weak labeling of the surrounding exocrine tissue. Not counterstained. Bar = 20 μm.

Hypophysis. Vibratome sections of the pituitary gland containing the anterior lobe, the pars intermedia, and the posterior lobe, immunostained with anti-NCAM antibodies, demonstrated marked differences in staining intensity of these three areas (not shown). In the anterior hypophysis, all secretory cells were intensely surface labeled. Blood vessels were particularly visible in otherwise uncounterstained sections. In the intermediate lobe, cells could be discerned by their surface membrane staining, but the intensity of this labeling was considerably less than that in the anterior lobe. The neural lobe was very intensely stained, although it proved difficult to identify cell features with certainty.

Ultrastructural observations confirmed the surface labeling of all secretory cells in the anterior lobe (not shown; see Langley et al., 1987). The plasma membrane of pituicytes was also positive

Figure 5. Electron micrograph of pancreatic islet stained for NCAM as in Figure 4, illustrating intense surface labeling of several pancreatic B-cells. No detectable levels of the antigens are found intracellularly. Section not counterstained. Bar = 1 μm.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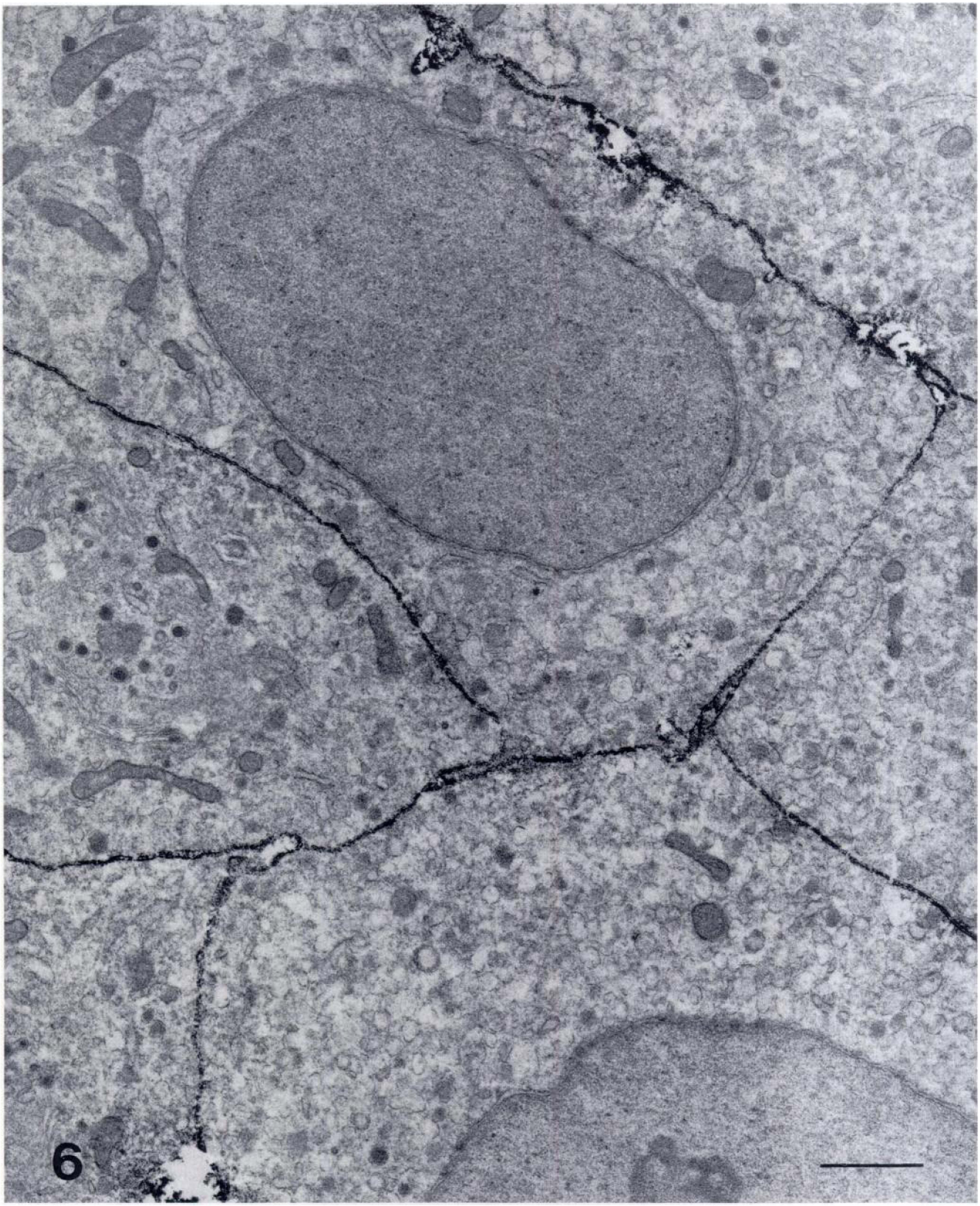


Figure 6. Intermediate lobe of rat hypophysis immunostained for NCAM. Reaction product is limited to the plasma membranes of secretory cells. Section not counterstained. Bar = 1 μm.

for NCAM. Basement membrane components, including collagen fibrils, were more weakly stained but undoubtedly positive. This extracellular staining was slightly less intense when primary antibodies were diluted 1:3000, while cell membrane staining remained very intense.

In the intermediate lobe, MSH (melanophore-stimulating hormone) cells were readily distinguished by their relatively limited number of small secretory granules. All secretory cells of this lobe were clearly labeled on their surfaces (Figure 6). In addition, fine interdigitated cell processes between cell bodies were surface labeled. The staining was, however, more discrete than that seen in the anterior lobe.

In the neurohypophysis (Figure 7), staining of the surface of all cell processes, including the terminal swellings of secretory cells containing granules or both granules and vesicles typical of synaptic vesicles, was strikingly intense. Frequently encountered pituicytes, recognized by the presence of large electron-dense "lipid droplets" in their cytoplasm, and their cell processes were also intensely surface labeled. No significant levels of intracellular staining were detected, but extracellular staining similar to that observed in the anterior lobe was apparent.

In control preparations none of the specific staining described above was observed, including staining of extracellular matrix components.

Immunochemistry

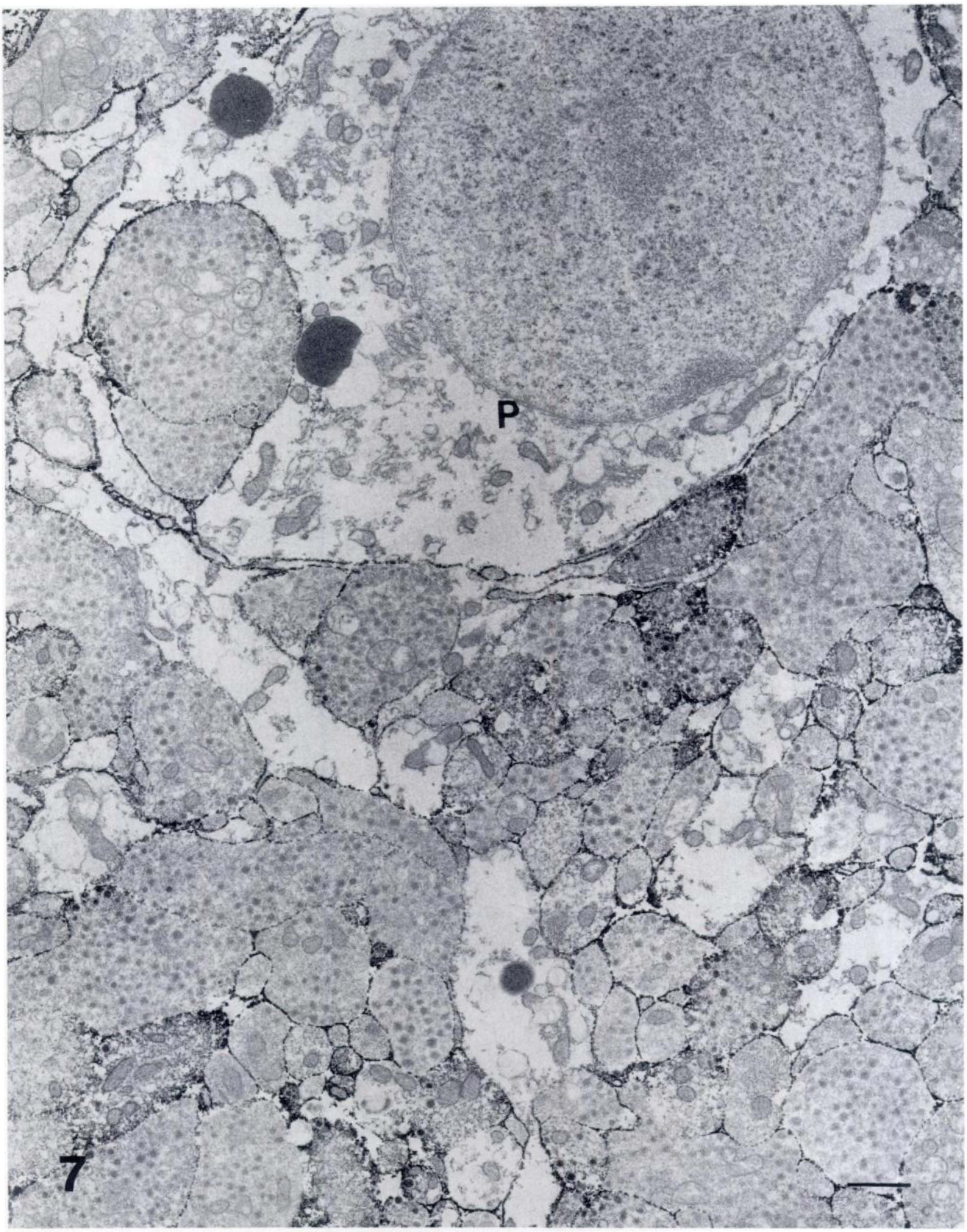
Immunoblots of cerebellum stained with the polyclonal anti-NCAM antibodies revealed the presence of three polypeptides of M_r 180, 140, and 120 KD (Figure 8a). Extracts of the neurohypophysis (Figure 8b) contained three high molecular weight bands: the most prominent component of 180 KD, the less intensely stained 140 KD determinant, and low levels (<5%) of the 120 KD polypeptide. In addition, a low molecular weight band (49 KD) was detected in neurohypophyseal extracts and also in the other tissues analyzed. The nature of this polypeptide was further characterized using immunoaffinity-purified antibodies against either the 49 KD peptide or the high molecular weight NCAM polypeptides. When tested on nitrocellulose transfers of cerebellar extracts, immunoblot-purified antibodies against the 49 KD peptide from pancreatic extracts (which contained the highest levels) did not crossreact with NCAM-180, -140, and -120. Likewise, antibodies eluted from high molecular weight cerebellar NCAM did not react with the 49 KD band on transfers of adenohypophyseal extracts, but they reacted with NCAM-140 of adenohypophysis. These results indicate that the polyclonal anti-NCAM antiserum contained an antibody population against the 49 KD peptide in addition to specific anti-NCAM antibodies. The nature of this peptide was further elucidated by washing tissue extracts before solubilization. Such washing considerably reduced the levels of the 49 KD band detected on blots with polyclonal anti-NCAM serum. When purified rat IgG was immunoblotted with the polyclonal serum, an identical band migrating at 49 KD was detected. It can therefore be concluded that the polyclonal serum contained contaminating antibodies reacting with the heavy chain of immunoglobulin. The antiserum was produced with NCAM antigen immunoaffinity-purified using a monoclonal antibody against NCAM, linked to Sepharose with cyanogen bromide. It is likely that small amounts of immunoglobulins were released with the antigen, eluted at low pH, and were subsequently injected with the antigen to produce the polyclonal serum. Since it can be concluded that the 49 KD peptide is not an NCAM, as was previously suspected (Langley et al., 1987), only specific anti-NCAM-reactive proteins (NCAM-180, -140, and -120) were quantitated (Table 1). The neurohypophysis was found to contain about 66% NCAM-180 and was therefore even richer in this variant than the cerebellum (Figure 8b; Table 1).

In immunoblots using whole rat pancreatic tissue, only a faint NCAM-140 band could be observed. This is probably due to the small amount (about 2%) of endocrine tissue within the pancreas. A considerable enrichment of NCAM-140 was found when islets obtained by microdissection (see Materials and Methods) were used for the immunoblots (Figure 8c). In addition to the prominent NCAM-140 besides the 49 KD band present in all tissue extracts, a further immunoreactive band was observed which was ascribed to proteolytic breakdown of NCAM during microdissection. Therefore, pancreatic islets are characterized mainly by the presence of NCAM-140 and thus resemble adrenal medulla and adenohypophysis, which also predominantly express this form.

By contrast, immunoblots of the insulinoma cell line (RIN A2) were characterized by the presence of two immunoreactive bands (Figure 8d). This tumor cell line mainly expressed the 140 KD variant, but lower levels (18%; see Table 1) of the 180 KD component were also observed. The pattern of immunoreactive bands displayed by the insulinoma cell line was very similar to that seen in PC12 cells (Table 1) (Langley et al., 1987; Friedlander et al., 1986).

Discussion

The data presented here on the expression of NCAM by endocrine cells extend our previous immunocytochemical studies on chromaffin cells and raise two new aspects which merit more detailed discussion. The first concerns the nature and the embryological origin of cells found to be immunoreactive with anti-NCAM antibodies; the second concerns the nature of the NCAM antigenic determinants present in the different endocrine tissues and cell lines examined. Early studies on NCAM concentrated on its expression by cells of the central nervous system and led to the general conclusion that, although the cell distribution is widespread in early embryonic life (Thiery et al., 1982), NCAM expression is much more restricted in adult tissues, being mainly limited to neural cells. Indeed, the expression of NCAM by many different types of neurons and glial cells in the adult is now well catalogued (Covault and Sanes, 1986; Van den Pol et al., 1986; Langley and Aunis, 1984; Langley et al., 1982,1983; Rutishauser et al., 1978a, b). Notable exceptions to this generalization for normal adult tissues include the expression by skeletal muscle cells at the neuromuscular junction (Covault and Sanes, 1986), expression by feather follicles in adult chickens (Chuong and Edelman, 1984), and the suggestion that chick lung epithelium retains NCAM expression in adulthood (Crossin et al., 1985). In considering the results obtained for the endocrine cells examined in this study, it is important first to underline two principal features that distinguish them from each other. The two cell lines are the result of malignant transformations and the endocrine tissues differ in their embryological origins. Although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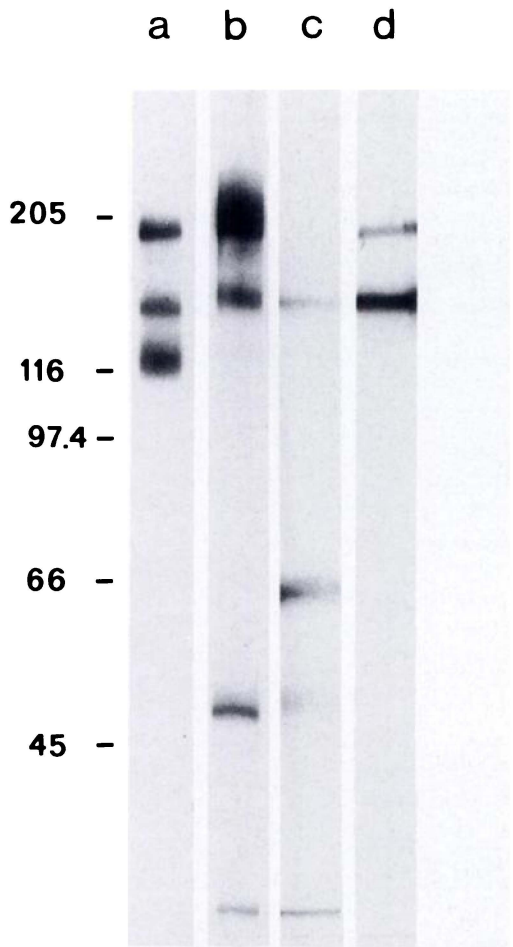

Figure 8. Immunoblots of the NCAM determinants in cerebellum (**a**), posterior lobe of the hypophysis (**b**), pancreatic islets (**c**), and RIN cells (**d**). Extracts of rat tissues and cells (50–100 μg protein) were resolved by 7.5% PAGE, transferred to nitrocellulose, and reacted with rabbit anti-NCAM antibody. Binding of antibody was detected by radiolabeled protein A and visualized by autoradiography (see Materials and Methods). Molecular masses (KD) of marker proteins are indicated.

Table 1. *Relative proportions of NCAM polypeptides in different tissues and cell-lines*[a]

Tissues/cell lines	Molecular weight (KD) 180	140	120	*N*
Cerebellum	30.1 ± 6.9	28.6 ± 5.6	41.3 ± 5.2	7
Neurohypophysis	66.3	28.9	4.8	1
Adenohypophysis[b]	*	100	–	7
Adrenal medulla[b]	*	100	*	4
Pancreatic islets	–	100	–	2
PC12 cells[b]	18.9 ± 6.5	81.1 ± 6.5	–	4
RIN cells	18.3 ± 6.3	81.7 ± 6.3	–	7

[a] Extracts of the tissues and cells were prepared as described for Fig. 8. The resulting autoradiographs were scanned and the relative amounts of the NCAM immunoreactive bands (in percentage) were determined from peak areas (see Materials and Methods). None of the specific bands were immunostained in control transfers with normal rabbit serum instead of the polyclonal rabbit anti-NCAM serum at the same dilution. *N*, number of determinations. *Weak NCAM immunoreactive bands found only in overloaded gels (<10%).

[b] Corresponding immunoblots are given in Langley et al. (1987).

the nature of the secretory products of different endocrine cells is characteristic for a given cell or endocrine tissue, the existence of a number of common properties led Pearse to include them in a general classification as APUD (amine precursor uptake and decarboxylation) cells (Pearse, 1968), and to subsequently suggest that all such cells shared a common (neural crest) origin (Pearse, 1969). Although many different endocrine organs are indeed derived from the neural crest, including the adrenal medulla, the neurohypophysis and, according to recent transplantation studies (Couly and Le Douarin, 1985), also the adenohypophysis, there is now good evidence that not all endocrine cells, notably those of the islets of Langerhans, share such an origin. It is generally agreed that these cells arise from pancreatic ducts, and transplantation studies have demonstrated that they are not derived from neural crest cells (Teitelman and Lee, 1987; Le Douarin, 1978). Therefore, among the endocrine cells studied here the pancreas and the pancreas-derived insulinoma can be distinguished from the others by their non-neural origin.

Neuronal and endocrine cells share several common intracellular protein constituents, including neuron-specific enolase, synaptophysin/P38, and chromogranins (see, for example, Ehrhart et al., 1986; Navone et al., 1986; Schmechel et al., 1978). The finding reported here that NCAM is expressed by many different endocrine cells represents an additional common property of endocrine cells, also shared by neurons and glial cells. Since pancreatic islets are not derived from the neural crest, the present results emphasize that, although the majority of cells that express NCAM in adult tissues are neural in origin, this is not a prerequisite for adult NCAM expression.

The limited extracellular staining in endocrine tissues reported here does not appear to be the result of methodological artifacts. Interpretation of immunocytochemical data on basement membrane staining is not always clear-cut. In particular, it is difficult to assert that no limited diffusion of the diaminobenzidine reaction product or its re-adsorption at sites other than antigenic sites has occurred (see Courtoy et al., 1983, for more detailed discussion). Nevertheless, cell adhesion molecules have previously been reported to be located extracellularly in certain tissues (Sanes et al., 1986). Since NCAM comprises a heparin binding site (Cole and Glaser, 1986; Cole et al., 1986) capable of binding NCAM to collagen fibrils by the intermediary of laminin, it is reasonable to suppose that NCAM determinants lost from the cell surface would adhere to neighboring basement membrane components.

Analysis of the pattern of NCAM polypeptides reveals interest-

Figure 7. Neural lobe of rat hypophysis labeled with anti-NCAM antibodies. Note the intense plasma membrane labeling of both granule-containing terminal swellings and pituicytes (P). Occasionally intracytoplasmic reaction product is seen close to the surface membrane of terminal swellings. Section not counterstained. Bar = 1 μm.

ing differences between the different endocrine tissues examined, in addition to major differences from the normal adult brain pattern. Minor variations in the relative proportions of the three (180, 140, and 120 KD) polypeptides have been found between different regions of adult brain (Edelman, 1984; Rougon et al., 1982). The immunoblots of adult rat cerebellum included for comparison in the present study are typical of those reported previously. In contrast, the immunoblots of the endocrine tissues studied here differ from those of brain by the absence of certain typical NCAM polypeptides, as has been reported for cultured adrenal medullary chromaffin cells (Nybroe et al., 1986). Among the endocrine tissues examined here, the neurohypophysis had an NCAM pattern most closely resembling that of brain, insofar as all three NCAM variants are expressed. The neuronal cell bodies, the terminals of which constitute the major part of the neurohypophysis, are situated in the paraventricular and supraoptic nuclei of the hypothalamus. The NCAM profile of their cell extensions can therefore be expected to be similar to that found for other brain areas. Nevertheless, levels of NCAM-120 are very low compared to CNS tissues. The relative simplicity of the NCAM pattern for adenohypophysis, adrenal medulla, and microdissected pancreatic islets was unexpected. It has been suggested that NCAM-140 is the first NCAM polypeptide to appear during early embryogenesis (Levi et al., 1987) and that NCAM-180 is characteristic of differentiated post-mitotic neurons (Pollerberg et al., 1986). This suggests that endocrine cells of these tissues retain a more primitive NCAM phenotype, whereas neurohypophyseal cell processes (with true neural origin) express a more typically neuronal pattern. The relative increase in NCAM-180 in two tumour lines studied so far (PC12, RIN A2), compared with their in situ counterparts, is intriguing and may indicate that their malignant transformation is associated with up-regulation of mRNAs coding for this component (see also Prentice et al., 1987). Therefore, although all the endocrine cells examined gave similar immunocytochemical staining with anti-NCAM antibodies, they differ considerably in the way they synthesize or process the NCAM antigens. Caution should be expressed in comparing blots because of possible variations in transfer efficiency of different polypeptides and the non-linear response of autoradiography. However, it is intriguing to note that, according to their NCAM phenotype (i.e., their NCAM polypeptide patterns), it is possible to distinguish between tumor cells and the tissues from which they are derived, and to differentiate neurohypophyseal tissue from other endocrine tissues, permitting a subclassification of endocrine cells.

The fact that discrete bands, corresponding to the sizes of NCAM polypeptides found in adult brain, were detected on immunoblots of endocrine tissues is indicative that the sialic acid content of endocrine NCAM is relatively low compared with that of the highly sialylated (and less adhesive) (Rutishauser et al., 1985) embryonic form of this molecule. Nevertheless, the band corresponding to NCAM-180 in the neurohypophysis was broader than expected, which may indicate slightly higher levels of sialic acid. This suggests that post-translational modifications of NCAM may not be identical in all endocrine tissues. Immunocytochemical studies of developing endocrine tissues with antibodies directed against highly sialylated forms of the molecule should demonstrate whether embryonic endocrine cells, like neurons, express NCAM containing high levels of polysialic acids, with lower adhesivity than their adult counterparts.

The different NCAM polypeptides present in the brain result from alternative splicing of a single NCAM gene (Murray et al., 1986), producing several mRNA species. The regulation of this process resulting in the different NCAM patterns of the tissues and cells investigated here is not known. Further immunochemical and immunocytochemical studies, combined with analysis of mRNAs coding for NCAM, may provide insight into cell adhesion phenomena involved in histogenesis of the endocrine tissues.

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