Endocrine cells share expression of N-CAM with neurones

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The expression of the neural cell adhesion molecule, N-CAM, was examined in the anterior lobe of rat hypophysis by immunocytochemistry at light and electron microscope levels. In addition, N-CAM antigenic determinants present in adrenal medulla, anterior hypophysis and PC12 cells were compared by immunoblotting with those found in cerebellum. All secretory cells in the anterior hypophysis were found to be N-CAM positive on their surfaces, but not all of the three polypeptide determinants typical of cerebellum were present in the endocrine tissues or cell line tested. In addition, a new N-CAM determinant of 49 kDa not present in cerebellum was found in adrenal medulla and hypophysis, although it was absent from PC12 cells. The possible implications of these data are discussed.

Neural cell adhesion molecule; Hypophysis; Immunocytochemistry; Immunoblotting; (Adrenal)

1. INTRODUCTION

The recent explosive interest in cell adhesion molecules (CAMs) is a direct consequence of the fundamental roles they are thought to play during early embryogenesis and tissue formation [1,2]. The most well known of them, studied independently under the names of N-CAM [3] (neural-CAM), D2 protein [4] and BSP-2 [5], has been shown to consist in brain of a family of three glycoproteins of M_r 180000, 140000 and 120000 [6,7] which are implicated in neurone-neurone adhesion by a homophilic binding mechanism [8,9]. While N-CAM was originally considered to be limited to neurones in adult tissues, ultrastructural immunocytochemical studies have since provided unequivocal evidence that glial cells, both astrocytes [6,10] and Schwann cells [11], also express N-CAM (see also [12,13]). Apart from a very limited expression by skeletal muscle at the neuromuscular junction [14], its expression in the adult

has been largely though not exclusively considered to be limited to nervous tissues. N-CAM has been found in certain cells outside the nervous system (e.g. chromaffin cells in the adrenal medulla [11]) but such cells are derived from the neural crest. Here we extend our previous observations on endocrine cells in the adrenal gland and investigate the possible expression of N-CAM by other endocrine cells which have a non-neural origin. The present results indicate a much wider distribution of N-CAM in adult tissues than has previously been supposed. N-CAM is shown by immunocytochemistry to be expressed by several endocrine cells of non-neural origin. Immunochemical data confirm the presence of N-CAM determinants typical of brain in endocrine cells although the relative proportions differ markedly. In addition, in two of the tissues examined a lower molecular mass N-CAM positive polypeptide was also detected.

2. EXPERIMENTAL

2.1. Immunoblots
Rat tissues were homogenized and PC12 cells

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lysed in 62.5 mM Tris/2% SDS. Homogenates and extracts were centrifuged at $13\,000 \times g$ for 4 min. Aliquots of the supernatants (70–100 μg protein) were separated on a 7.5% polyacrylamide gel in the presence of SDS under reducing conditions and transferred to nitrocellulose. Anti-N-CAM antiserum (kindly supplied by C. Goridis) was used at a 1:1000 dilution. The binding of antibody was detected using radiolabelled protein A.

2.2. Immunocytochemistry

Adult rats were perfused (½ h) with 4% formaldehyde in phosphate buffer (0.12 M) and excised pituitary glands were post-fixed in the same solution for a further 2 h. Immunocytochemistry was performed on 50 μm thick vibratome sections using the indirect immunoperoxidase method. Sections were preincubated in 5% normal sheep serum in phosphate-buffered saline (1 h) before incubation overnight at 4°C in rabbit polyclonal anti-N-CAM antiserum diluted 1:1000. Peroxidase-conjugated sheep anti-rabbit IgG diluted 1:100 was used as second antibody. Bound peroxidase was revealed with diaminobenzidine (0.05%) and hydrogen peroxide (0.01%) for 4 min at 20°C in the dark. Sections were photographed without counterstaining. For electron microscopy sections were subsequently osmicated (1% osmium tetroxide in 0.1 M sodium cacodylate, pH 7.2) dehydrated and flat embedded in Spurr resin. Thin sections cut parallel to the surface of the vibratome sections were viewed uncounterstained with a Philips EM 420 operated at 60 kV with a 20 μm objective aperture.

3. RESULTS AND DISCUSSION

N-CAM determinants were analysed in four different rat tissues or cell lines by SDS-polyacrylamide gel electrophoresis and subsequent immunoblotting with anti-N-CAM. Cerebellum was used as a reference tissue for comparison since its N-CAM species composition is well documented [6,7]. Adrenal medulla was chosen as an example of an endocrine tissue derived from the neural crest and PC12 cells were included as an example of a tumour cell line derived from a rat adrenal pheochromocytoma. The anterior lobe of the pituitary gland represents a second endocrine tissue of different origin containing several dif-

ferent types of secretory cell. In this study new immunohistochemical data provide information on the cellular distribution of N-CAM in the anterior lobe of the rat hypophysis at the light and electron microscopical levels to complement such data on cerebellum, adrenal medulla and PC12 cells reported elsewhere [10,11,15].

Comparison of immunoblots of extracts of the endocrine tissues and PC12 cells with those obtained with cerebellum (fig.1) show that some N-CAM positive bands typical of brain are also found in other tissues. In cerebellum, the three polypeptides previously shown to be immunoprecipitable with anti-N-CAM antibodies [5,16] were detectable; their intensities on radiolabelled blots were approximately equal. In contrast, immunoblots of PC12 cell extracts showed principally a band of 140 kDa with a less prominent band of 180 kDa but no polypeptide equivalent to the 120 kDa N-CAM glycoprotein, in agreement with recent data [17]. Only the 140 kDa band of the three cerebellar glycoproteins was detected in adrenal medulla extracts while an additional low-molecular-mass band of approx. 49 kDa was found. This latter band was very prominent and more intense than the 140 kDa band. Similarly, extracts of the anterior lobe of the hypophysis contained only these two N-CAM positive polypeptides at 140 and 49 kDa. In control immunoblots with normal rabbit serum replacing the specific rabbit anti-N-CAM serum, no specific labelling at 180, 140, 120 or 49 kDa occurred.

Light microscopy of vibratome sections of formaldehyde-fixed anterior hypophysis immunoperoxidase labelled for N-CAM shows intense surface staining of all secretory cells constituting the tissue (fig.2A). Blood vessel walls could also be clearly distinguished in otherwise uncounterstained sections. The staining pattern was identical whether the indirect or peroxidase-anti-peroxidase method [18] was employed and was also the same with three different polyclonal sera (two obtained using mouse N-CAM as immunogen, kindly provided by Dr C. Goridis and another obtained against bovine N-CAM, a generous gift from Dr G. Rougon). Controls employing normal rabbit serum gave low background but none of the specific staining characteristics of anti-N-CAM. Electron microscopy (fig.2B) of anti-N-CAM labelled sections confirmed the distribution of antigen over the en-
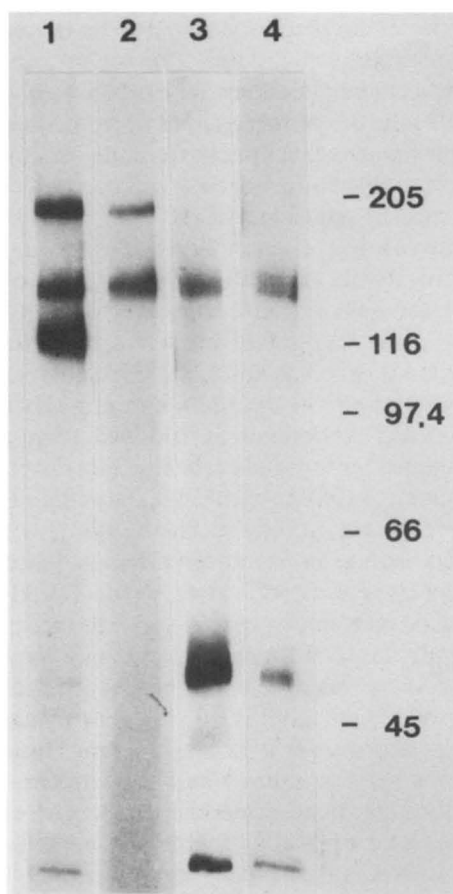
Fig.1. Immunological identification of N-CAM in cerebellum (lane 1), pheochromocytoma cells (PC12 cells; lane 2), adrenal medulla (lane 3) and anterior lobe of the hypophysis (lane 4). Molecular masses (kDa) of marker proteins are indicated.

tire surface of all of the different types of secretory cell. No appreciable level of intracellular labelling was noted. In addition, immunoperoxidase reaction product was found extracellularly and in particular associated with basement membrane elements such as collagen.

Immunocytochemical evidence suggests that N-CAM determinants are expressed over the entire surface membrane of neurones both in culture [19] and in vivo [10] and in addition on the astrocyte surface [11]. An intracellular location has been reported only in cerebellar granule cells and astrocytes [10]. PC12 cells have been previously shown to express N-CAM on their surface [15]; N-CAM has been found to be limited to the plasma mem-
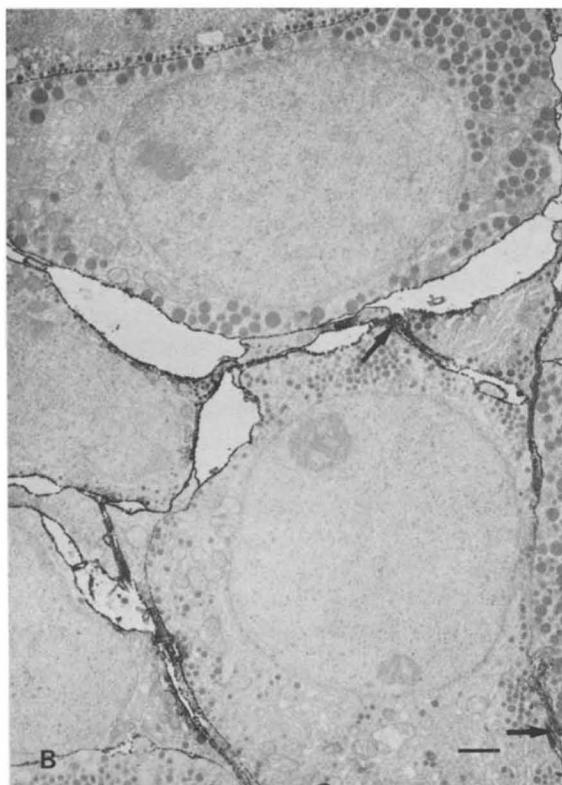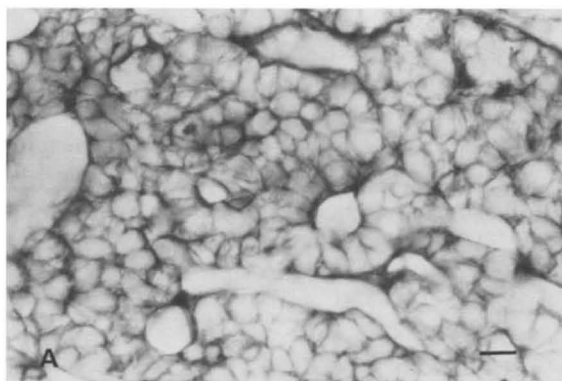

Fig.2. Adult rat pituitary anterior lobe immunoperoxidase labelled for N-CAM. In the optical micrograph (A) apparently all cells in the tissue are surface labelled. In the electron micrograph (B) it is evident that reaction product is limited to the surface of all types of secretory cells. No intracellular staining is detectable but some labelling of extracellular matrix components (including collagen and the basement membrane) is apparent (arrows). Sections not counterstained. Bar, 20 μm (A); 1 μm (B).

branes of both adrenergic and noradrenergic cultured adrenal medullary chromaffin cells [20], and that of Schwann cells [11]. No analysis of the N-CAM glycoprotein pattern has previously been available for the adrenal gland and neither immunochemical nor immunocytochemical data on the anterior pituitary have been published to date. The results of the two approaches employed here lead to the conclusion that adult endocrine tissues or cell lines derived from them should be added to the above list of cells that express N-CAM. However, three N-CAM glycoproteins are typical of adult cerebellar tissue: a more restricted number is found in endocrine tissues. Only two determinants were detected in PC12 cells and only one of the species typical of brain was found in adrenal medulla and the anterior lobe of the hypophysis. It is not surprising that the 120 kDa glycoprotein was not detectable in PC12 cells since chromaffin cells in situ from which they derive do not appear to express it. Other tumour cell lines also show a limited expression of the N-CAM determinants [21]. Although it is accepted that the relative proportions of the three glycoproteins vary both with age and with brain region [7], it is of particular interest that the 180 and 120 kDa polypeptides were absent from the two endocrine tissues examined here while at the same time an additional N-CAM positive band of 49 kDa was prominent. It seems unlikely that this represents an unrelated polypeptide cross-reacting with the antiserum since three separate antisera gave identical results. It is probable that this represents a specific degradation product of the typical N-CAM determinants.

While N-CAM appears to be widely distributed in early embryos [22-24], it is generally accepted that its expression in the adult is essentially restricted to neural cells, though N-CAM has been reported to persist in lung epithelium in the adult chicken [23]. The present data clearly indicate that N-CAM is expressed by many endocrine cells in the adult. The anterior lobe of the hypophysis arises from an invagination of the epithelium of stomoderm which forms Rathke's pouch [25]. Elegant transplantation studies performed on chick embryos at very early development stages [26] suggest that Rathke's pouch is embryologically derived from the anterior neural ridge. The adenohypophysis may thus be considered to be a neural tissue. Whether the stomodeal epithelium expresses N-CAM at the time of early histogenesis of the pituitary gland remains to be examined. However, when cellular aggregation occurs to form the future hypophysis it would appear that cells destined to become the endocrine cells of this tissue undergo an induction process, which may also be responsible for switching on N-CAM expression. Such a process could be analogous to the latter stages of adrenal medulla histogenesis in which N-CAM negative cells migrating from the neural crest re-express N-CAM at the time of chromaffin cell aggregation. The data reported here support the view that N-CAM expression in adult tissues reflects histogenetic requirements to stabilize cells into aggregates, to stabilize side-to-side cellular contacts (as has been proposed for cerebellum [10]) or to stabilize specific contacts between two different cell types (as at the neuromuscular junction [14]). Thus, these cell adhesion molecules may be at least in part responsible for the aggregation of the cells in the anterior hypophysis which simultaneously develop endocrine characteristics. A developmental study of N-CAM in the hypophysis will help to elucidate both the timing of its expression by cells destined to constitute the pituitary endocrine gland and its likely role in the formation of this tissue.

ACKNOWLEDGEMENT

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