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Adrenal chromaffin granules and secretory granules from thyroid parafollicular cells have several common antigens

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The presence of various antigens in two types of isolated endocrine vesicles (chromaffin granules and secretory vesicles of thyroid parafollicular cells) was investigated by immunoblotting. The two types of vesicles have three common secretory proteins: chromogranin A, chromogranin B and secretogranin II. Furthermore, six common membrane antigens were found: cytochrome *b*-561, carboxypeptidase H, glycoprotein II, glycoprotein III, synaptin/synaptophysin and SV 2. These results demonstrate that vesicles obtained from neural crest-derived endocrine cells not only share several common secretory peptides and proteins, but also have common properties as far as their membrane antigens are concerned.

Chromaffin granule; Chromogranin A; Chromogranin B; Synaptin; Synaptophysin; Parafollicular cell thyroid; Cytochrome *b*-561

1. INTRODUCTION

The biochemical composition of the chromaffin granules, the catecholamine-storing organelles of adrenal medulla, has been characterized in great detail [1–3]. All the major protein components of these vesicles have been isolated, immunologically characterized and for some of them, the primary amino acid sequence has been elucidated. Less is known about secretory vesicles of other endocrine organs, mainly because of the difficulties encountered in isolating them in high purity and large quantity. Thus, purified preparations of the calcitonin-storing vesicles of thyroid parafollicular cells have only recently become available [4,5]. In the present study, we establish that these vesicles have at least nine proteins in common with adrenal chromaffin granules.

2. MATERIALS AND METHODS

A washed large granule fraction was obtained from sheep adrenal medulla [6]. The secretory granules of sheep parafollicular cells were isolated following the procedure of Barasch et al. [4] with slight modifications. Fresh lamb thyroid glands were homogenized in 0.32 M sucrose containing Hepes (5 mM, pH 7.0), EDTA (5 mM), monothioglycerol (5 mM), pepstatin (2 μg/ml) and leupeptin (5 μg/ml). A crude granule pellet obtained from this homogenate was purified by two successive metrizamide gradients [4]. The purified vesicles were obtained from the 18/28% metrizamide interlayer. This fraction was diluted 7 times with homogenization buffer and centrifuged at 9700 × *g* for 20 min. The resulting pellet was lyophilized and used for immunoblotting, which was performed as previously described in detail [7]. The following rabbit antisera were used: anti-bovine chromogranin A and B [8], secretogranin II (chromogranin C, [9]), anti-bovine cytochrome *b*-561 (kindly provided by D.K. Apps, Edinburgh), anti-carboxypeptidase H raised against the synthetic peptide (SF 24) representing the carboxy terminus of the enzyme [10], anti-bovine glycoprotein II [11], anti-bovine glycoprotein III [12], and anti-rat synaptin/synaptophysin (kindly provided by R. Jahn). Anti-SV2 was a monoclonal antibody (10 H3, kindly provided by E. Floor).

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3. RESULTS

In immunoblotting, the antisera raised against bovine and rat antigens gave a good cross-reaction with the proteins present in sheep chromaffin granules (fig.1). They were, therefore, suitable to test for the presence of these antigens in secretory granules isolated from sheep parafollicular cells. These latter organelles apparently contain the three secretory proteins also present in chromaffin granules, i.e. chromogranins A and B and secretogranin II (for nomenclature see [13]). These secretory proteins are processed within the vesicles by endogenous proteases, therefore they are present as several immunostained bands [2]. In the parafollicular granules, the proteolytic processing is apparently more marked, since the proprotein, i.e. the slowest moving band of chromogranin B, is relatively reduced and that for secretogranin II is practically ab-

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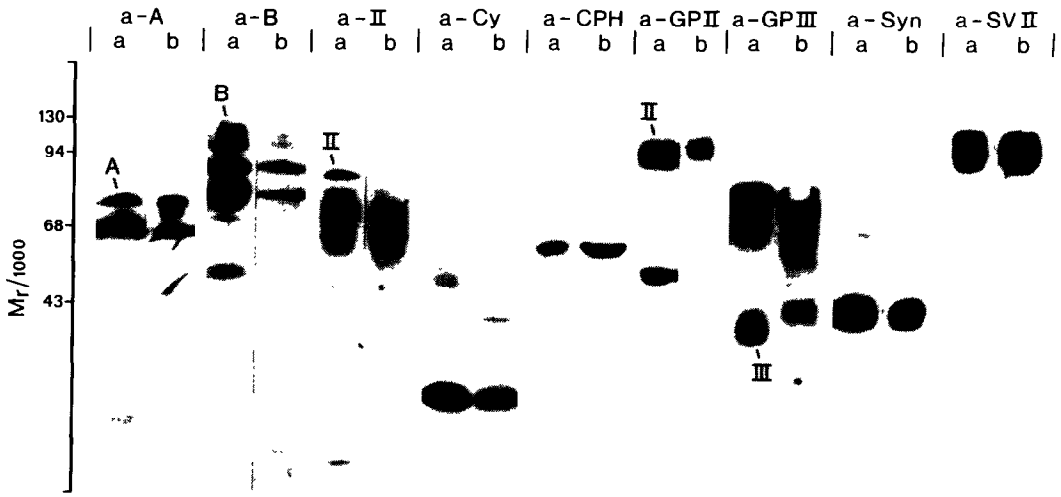

Fig.1. Immunoblot analysis of proteins in sheep adrenals and secretory granules of parafollicular cells. Large granules from sheep adrenals (a) and secretory granules of parafollicular cells (b) were subjected to one-dimensional immunoblotting. The antisera against chromogranin A, B and secretogranin II (a-A, a-B, a-II) react with the proproteins (marked A, B and II for the adrenal medulla) and several endogenous breakdown products. Faint immunostained bands in the case of anti-cytochrome (a-Cy) are polymerization products of this protein. The anti-carboxypeptidase H antiserum (a-CPH) reacts with one major component which is also present in bovine chromaffin granules [29], an additional slower moving band in sheep adrenals is likely to be a precursor since this enzyme is synthesized as a large proprotein [10]. The antiserum against glycoprotein II (a-GPII) reacts in sheep adrenal granules with an additional faster moving band, which was not found in bovine chromaffin granules [11]. The antiserum against glycoprotein III (a-GPIII) is not specific, since it is also immunostaining the chromogranins A. The band representing GPIII is marked by III.

sent. The two types of secretory granules also have several common membrane antigens (fig.1): cytochrome *b*-561, carboxypeptidase H, glycoprotein II and glycoprotein III, synaptin/synaptophysin (for nomenclature see [14]) and SV2. In both vesicle types, the antigens migrated identically with the exception of glycoprotein III, which moved slightly slower in parafollicular granules. In the absence of reducing agents, glycoprotein III migrates as a dimer [12]. An experiment under such conditions (results not shown) demonstrated that the immunoreactive bands at 43000 kDa of both types of vesicles had disappeared due to dimerization.

4. DISCUSSION

The present study establishes that secretory organelles from two different endocrine organs have a rather large number of antigens in common. This was not so surprising for the chromogranins. It is by now well established that these secretory peptides have a widespread distribution in endocrine tissues [15–20]. Thus, chromogranin A has also been found in the parafollicular thyroid cells by immunohistochemistry [20] and in the secretory granules of medullary carcinoma derived from these cells by immunoelectron microscopy [21,22]. For chromogranin B and secretogranin II, the immunohistochemical results yielded only negative or variable data on normal tissue [20,23], but both antigens were found in thyroid medullary carcinomas [24]. Our present results with immunoblotting demonstrate unequivocally that chromogranin A, B and secretogranin II are present in the parafollicular cell granules.

In addition to these secretory proteins, these two types of endocrine cell organelles have several membrane antigens in common. Two of them have a well-established function, i.e. cytochrome *b*-561 and carboxypeptidase H. The cytochrome has already been found in several endocrine organs, however the parafollicular cells of the thyroid gland were not investigated [25,26]. Carboxypeptidase H has been previously demonstrated in chromaffin granules, in the pituitary gland and in brain [27–29], it is now also found in the parafollicular cell granules. This enzyme is involved in the processing of peptides, whereas the cytochrome can provide electrons for an ascorbic acid cycle mediating the action of monooxygenases like the peptidyl glycine alpha-amidating monooxygenase [30]. This indicates that the C-cell granules might be involved in posttranslational peptide processing. The now established presence of the chromogranins and the previous finding of somatostatin [31] in these vesicles is consistent with such a function. Four additional antigens which are also found in both types of vesicles have not yet any established function. Glycoprotein III has also previously been found in anterior pituitary [12] and glycoprotein II has been found in vesicles of both endocrine and exocrine tissues [11]. Synaptin/synaptophysin was originally described in synaptic vesicles [32,33], but is also present in chromaffin granules [32,34,35]. Analogous findings were reported for the glycoprotein SV2 which has been demonstrated in brain and several endocrine tissues [36].

It is by now a well-established fact that several peptides and proteins are present in the secretory content of endocrine vesicles, in addition to the respective hormones. These peptides have a widespread distribution in the endocrine system. This is true for the neuropeptides [37] and also for the chromogranins [2], which in any case may be precursors of active peptides. Parafollicular cells and the adrenal medullary cells are both derived from the neural crest [38] and both can be induced to extend neurites when cultured with nerve growth factor [39]. Although it is possible that the analogous properties of parafollicular and chromaffin granules reflect their related ontogeny, on the basis of present and previous studies we would like to propose that similar common properties may be found for the membranes of all endocrine vesicles. Some membrane antigens will be quite specific for one type of vesicle, e.g. proteins like dopamine β-hydroxylase which are involved in the synthesis of a special transmitter. Others, e.g. those involved in the processing of peptides (carboxypeptidase H and possibly cytochrome *b*-561) may have a widespread distribution in analogy with the peptides themselves. Those antigens like glycoprotein II, synaptin/synaptophysin and SV2 which are widespread will certainly have a yet to be discovered relevant function. In any case, our study has already established that the makeup of the membranes of two types of secretory vesicles have at least six antigens in common. Such similarities are likely to apply to further vesicles and will certainly include even additional antigens.

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