A high ratio of chromogranin A to synaptin/synaptophysin is a common feature of brains in Alzheimer and Pick disease

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Chromogranin A and synaptin/synaptophysin were characterized by immunological methods in human autopsy brain tissue from patients with Alzheimer's and Pick's disease. In immunoblots there was no qualitative difference between the antigens in control and diseased brain, but significant quantitative differences were found. In all Alzheimer cases there was a significantly lower level of synaptin/synaptophysin, whereas chromogranin A was higher in 4 out of 5 cases and in all cases relative to synaptin/synaptophysin. An analogous finding was obtained for Pick's disease. Immunohistologically a consistent staining of neuritic plaques for chromogranin A, but not for secretogranin II was found in Alzheimer cases. In Pick's disease the characteristic Pick bodies showed an analogous specific immunostaining.

Alzheimer's disease; Pick's disease; Chromogranin A; Secretogranin II; Synaptin/synaptophysin

1. INTRODUCTION

Chromogranin A and secretogranin II (chromogranin C) are proteins present in endocrine [1,2] and in large dense core vesicles of brain [3]. Synaptin/synaptophysin [4] is found in high concentrations in synaptic vesicles [5-7], but also in large dense core vesicles and endocrine vesicles [5,8,9]. Recently it has been reported that a monoclonal antibody against chromogranin A recognizes the characteristic plaques of Alzheimer's disease [10] indicating the presence of chromogranin immunoreactive material in these lesions. We report now for Alzheimer's and Pick's disease that a high chromogranin A/synaptin(synaptophysin) ratio is a common characteristic for these two brain diseases leading to dementia.

2. MATERIALS AND METHODS

The study was performed on human autopsy brain tissue from the Vienna Prospective Longitudinal Study on Dementia [11]. It included 21 cases of Alzheimer's disease (age: 59–88), 3 cases of Pick's disease (age: 66–75), 4 cases of mixed dementia (multiple cerebral infarcts together with lesions of Alzheimer's type, age: 77–92), and 5 non-demented control cases (age 57–91).

Immunostaining for chromogranin A and secretogranin II was performed on paraffin sections. The sections were deparaffinized, unspecific binding was blocked with 10% fetal calf serum. Polyclonal rabbit primary antisera were used in dilutions of 1:300. Bound antibody was visualized with an alkaline-phosphatase/anti-alkaline phosphatase technique [12]. As controls the primary antiserum was omitted or replaced by normal rabbit serum in identical dilutions. The techniques of Bielschowsky silver impregnation and immunocytochemistry for tau were described in detail earlier [13].

For immunoblotting with a polyclonal antiserum [14] against human chromogranin (dilution 1:200) heat stable extracts (corresponding to 1 g wet tissue) of lyophilized brain tissue [15] were used. For synaptin/synaptophysin (polyclonal antiserum against rat synaptin/synaptophysin kindly provided by R. Jahn, Munich, FRG) lyophilized tissue samples (corresponding to 25 mg wet tissue) were dissolved directly in electrophoresis sample buffer.

3. RESULTS

In the normal human neocortex and hippocampus the distribution of chromogranin A-like immunoreactivity was identical to that described previously in sheep brain [3]. Within neurons, chromogranin A was found in a granular distribution in the cytoplasm of nerve cells (results not shown). No chromogranin A-like immunoreactivity was found in the white matter. Secretogranin II reactivity was identical to that of chromogranin A, although the overall intensity of the immune reaction was lower. In Alzheimer's disease, in addition to the pattern found in normal brains numerous senile plaques contained chromogranin A-reactive, dystrophic neurites. A detailed quantitative analysis of these plaques in comparison with those stained by Bielschowsky's silver technique revealed that, dependent upon the case, 2–100% (50 ± 5, n = 24) of plaques in the tissue visualized by the modified Bielschowsky technique contained chromogranin A like immunoreactivity. This highly divergent incidence was due to the fact, that chromogranin A was exclusively present in plaques with a neuritic component. Diffuse
Fig. 1. Immunoblots of tissue samples of control and Alzheimer brain. Immunostaining (anti-A: anti chromogranin A; anti-Syn: anti synaptin/synaptophysin) of samples from temporal cortex of brains from a control patient (C: 57 M), from two Alzheimer cases (AI: 75 M; A2: 79 F) and from a case with morbus Pick (P) are shown. A quantitative comparison of the various samples is not possible from this figure since the film obtained from the labelled blot was overexposed to show as many bands as possible in a way suitable for printing. PG-A: proteoglycan-chromogranin A; AM: heat stable extract from human adrenal medulla.

('early') plaques did not reveal chromogranin A immunoreactivity. In Pick's disease all Pick bodies (as determined by staining adjacent sections with either Bielschowsky silver impregnation or immunocytochemistry for tau) were intensively stained with antibodies against chromogranin A. In both Alzheimer's and Pick's disease secretogranin II like immunoreactivity was exclusively present in granular distribution in the cytoplasm of nerve cells. Pick bodies and plaque neurites were negative for secretogranin II. These immunohistochemical results demonstrate that chromogranin A immunoreactivity is consistently found both in Alzheimer plaques and Pick bodies; however, this method does not allow any statements on the properties and relative quantity of the immunoreactive material. In order to define this immunoreactive material in molecular terms immunoblots were performed. Previous studies (see [1]) have established that in extracts of immediately processed endocrine and brain tissues antisera against chromogranin A react with the proprotein chromogranin A and several smaller proteins which are formed within the storage vesicles by endogenous processing. For tissues not immediately processed a further breakdown leading to smaller proteins becomes apparent [16]. For human brain, as shown in Fig. 1, the proprotein chromogranin A can still be demonstrated (A in Fig. 1). In addition a faster moving breakdown product (A1 in Fig. 1) is present in varying relative amounts. A slower moving band (PG-A in Fig. 1) has been previously [17] identified as the proteoglycan form of chromogranin A, which can be found consistently in brain tissue, e.g. of rat [1,18]. As shown in Fig. 1 there is no significant qualitative difference between the chromogranin A-reactive bands in control or diseased brains. The apparent prominence of the proteoglycan-chromogranin A in Pick's brains is due to the fact that higher levels of total chromogranin were present in these samples. Synaptin/synaptophysin (see Fig. 1) could also be demonstrated in human brain. For secretogranin II our method was not sensitive enough.

The quantitative evaluation of chromogranin A and synaptin/synaptophysin levels is shown in Table I. A consistent finding for all Alzheimer cases was the lower level of synaptin/synaptophysin; on the other hand in 4 out of 5 Alzheimer cases the chromogranin A level was remarkably elevated. In a fifth case the chromogranin A level was below control, but the chromogranin A/synaptin(synaptophysin) ratio was still high. It seems likely that in this case a considerable cell loss has reduced a previously elevated chromogranin A level below control. Only one brain of Pick's disease was available for immunoblotting; nevertheless, several samples were analyzed and a very high level of chromogranin A and of the chromogranin A/synaptin(synaptophysin) ratio was detected.

4. DISCUSSION

Synaptin/synaptophysin is a protein found in high concentrations in synaptic vesicles [5–7], and in lower concentration in large dense core vesicles and in endocrine vesicles like chromaffin granules [5,8,9]. Its
consistent low levels in Alzheimer’s (which is in agreement with immunohistochemical data [19,20]) and Pick’s brains can simply be an expression of neuronal and synaptic loss occurring in these diseases, but a specific loss of synaptic vesicles should also be considered. On the other hand, chromogranin A levels are increased. This protein is found in endocrine vesicles and large dense core vesicles of brain and is secreted together with other constituents of these vesicles by exocytosis. This property is shared with neuropeptides [1]. For some neuropeptides, an immunohistochemical staining of Alzheimer plaques has been observed [21], but we are unaware of a report describing high levels of any of these peptides in brain. In fact reduced levels have been reported (see also [22]) for neuropeptide Y [23] and somatostatin [24]. In addition we have not found immunostaining of Alzheimer plaques or of Pick bodies with an antiseraum against secretogranin II which at least in chromaffin granules is colocolized with chromogranin A [25]. Therefore our results on chromogranin A levels seem quite specific for this secretory protein. Unfortunately the function of this protein is still not very well defined, although peptides derived from it (e.g. pancreastatin) have been shown to inhibit the secretion of hormones [26,27].

Elevated levels of chromogranin A in diseased brain might be a consequence of an increased synthesis or decreased secretion or breakdown. Our present study does not yet allow us to state whether chromogranin A is only accumulating in the typical lesions or is elevated in all neurons. This can only be elucidated in further studies including mRNA-measurements. In this context it is interesting to note that one report has claimed that in adrenal medulla of Alzheimer patients large vacuoles with electron dense content (protein?) have been found [28]. The adrenal medulla contains a high concentration of chromogranin A [1]. Does this suggest that peripheral organs also contain more chromogranin as it has recently been shown for the amyloid β protein precursor [29]? In any case we consider our results important for the following reasons: we have found chromogranin A immunostaining both in Alzheimer plaques and Pick bodies. The immunoreactive material was identified as chromogranin A. In both diseases the brain contains increased levels of this secretory protein found in large dense core vesicles whereas synaptin/synaptophysin levels are low. These data for chromogranin A appear specific, since other secretory constituents of large dense core vesicles (secretogranin II, neuropeptides) behave differently. Thus for these two diseases leading to dementia but with significantly differing pathohistology a high chromogranin A/synaptin(synaptophysin) ratio is a common and specific denominator. These data might also become relevant for diagnostic purposes: does the cerebrospinal fluid of Alzheimer and Pick patients contain higher levels of chromogranin A or of peptides derived from it?

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REFERENCES