12

Role of autoimmunity in hypothalamic disorders

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The proposition that the hypothalamus may be affected by organ or cell type-specific autoimmune diseases is based on recent findings which have indicated that this type of pathological mechanism could also be involved in certain forms of central diabetes insipidus (DI). This disease will therefore be emphasized in the discussion below.

Brain tissues such as the hypothalamus are separated from blood by the blood–brain barrier which consists of a capillary endothelial cell layer with tight junctions, a dense basement membrane and an almost complete layer of surrounding astrocytes with their protruding foot processes (Reese and Karnovsky, 1967; Brightman and Reese, 1969). Therefore, under normal conditions, the hypothalamus is protected from a variety of circulating cellular and humoral components of the blood. Molecules and cells cannot pass between the cells but they must penetrate the luminal and anteluminal cell membranes. Only some parts of the brain, the so-called circumventricular organs (Weindl, 1973) are excluded from this barrier.

Under pathological conditions, the intact blood–brain barrier may be disrupted, e.g. by invading tumours that are vascularized by fenestrated endothelia (Long, 1979). A reversible opening of the blood–brain barrier is observed in encephalitis, where even low initial doses of peripherally administered penicillin can produce therapeutic intracerebral levels of the antibiotic. The blood–brain barrier can also be reversibly opened by a number of experimental conditions such as bicuculline-induced seizures (Nitsch et al, 1986) or by intra-arterial infusion of hypertonic solutions that may cause shrinking of endothelia and lead to a disconnection of tight junctions (Neuwelt and Rapoport, 1984). The possibility thus exists of a contact of hypothalamic tissues with the peripheral immune system.

HYPOTHALAMIC AUTOIMMUNITY AND IDIOPATHIC CENTRAL DIABETES INSIPIDUS

Spontaneously acquired central DI is caused by a variety of underlying diseases which involve the hypothalamus. However, even when patients are repeatedly evaluated clinically, the cause of DI remains obscure in about

one-third of the cases (Blotner, 1958; Moses, 1985). A body of evidence has been accumulated suggesting that at least some of these cases are autoimmune in origin.

Demonstration of cytoplasmic vasopressin cell antibodies

For the detection of vasopressin cell antibodies, human fetal hypothalamus obtained within the first two hours of therapeutic abortion provides the best substrate (Table 1). Seven-micron cryostat sections are cut and arginine-vasopressin (AVP)-containing cells are histochemically localized by anti-AVP sera. The hormone keeps well-preserved for many hours after death (Emson et al, 1982) whereas the relevant autoantigens reacting with the sera from patients with idiopathic central DI are rapidly destroyed. Since the time of fetal death in utero is not known, it is therefore necessary to apply an AVP cell antibody-positive patient's serum before new samples can be reliably tested in a routine or research screening programme.

Table 1. Incidence of vasopressin cell antibodies on different tissues of hypothalamus in 10 positive cases.

Source of hypothalamus	Age of donor	Time until freezing (hours)	Lipofuscin contents	Vasopressin cell antibodies: number positive
Post-delivery				
Human fetal	21 weeks	2	—	10
	21 weeks	1.5	—	8
	18 weeks	1	—	6
Post-mortem				
Baboon adult	?	0.25	(+)	8
Human adult	50 years	3	+	4
	38 years	6.5	(+)	1
	91 years	2.5	+++	1

From Scherbaum et al (1985c), with permission.

Adult post-mortem tissue obtained several hours after death gives only poor results. Fresh baboon hypothalamus obtained from young animals may be used as a substitute for human fetal tissue (Table 1). Older adult donors, both humans and non-human primates are unsuitable because the natural accumulation of lipofuscin granules in secretory hypothalamic cells interferes with the reading of the specific cytoplasmic immunofluorescence given by the positive patient's serum (Figure 1).

AVP cell antibodies are tested by indirect immunofluorescence (IFL) on unfixed or acetone-fixed sections. Bouin's or formaline-fixed tissue is unsuitable for the assay since many false-negative and false-positive results are obtained. Cytoplasmic AVP cell antibodies are of IgG and/or IgA class. The application of both anti-IgG and anti-IgA or of polyvalent anti-gammaglobulin conjugates is therefore required for antibody testing. The titres range from 1:1 to 1:64. Half the antibodies also fix complement, but this feature is not correlated with a distinct clinical subgroup of patients with idiopathic central DI. The specific pattern of an AVP cell antibody-positive serum on baboon hypothalamus is given in Figure 2.

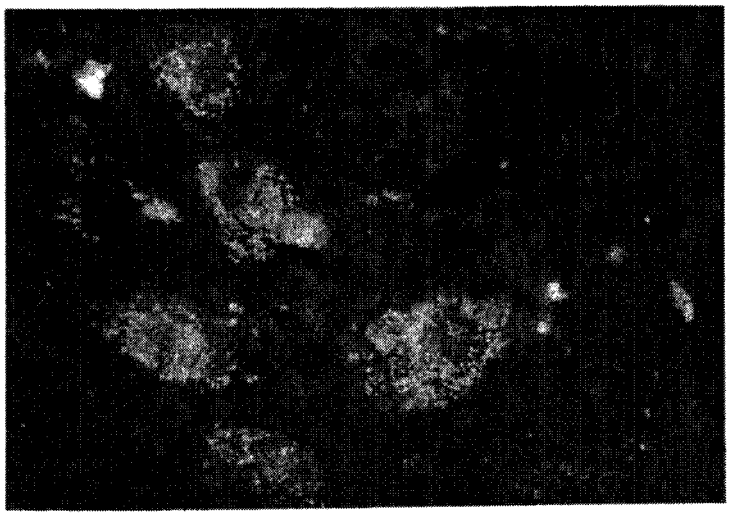

Figure 1. Cytoplasmic autofluorescence of granular lipofuscin on a cryostat section of baboon hypothalamus from an old donor. The section was incubated with normal human serum and stained with fluoresceinated (fluorescein isothiocyanate, FITC) goat anti-human IgG conjugate.

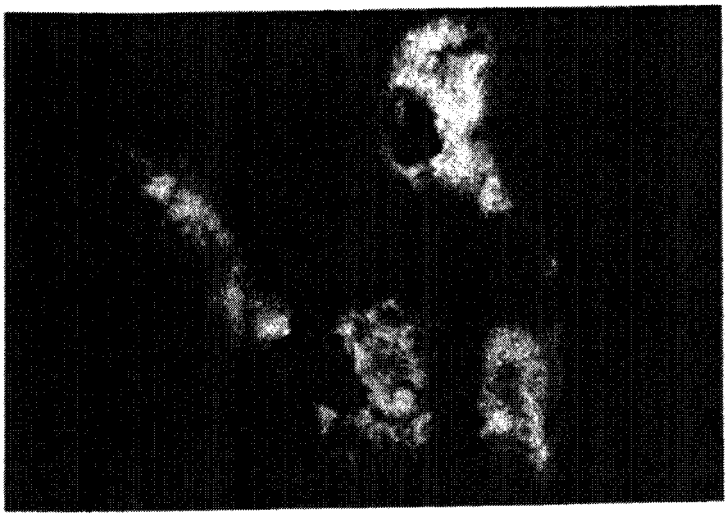

Figure 2. Specific immunofluorescence pattern of vasopressin cell antibodies demonstrated on fresh baboon hypothalamus from a young animal. The serum of a patient with 'idiopathic' central DI was incubated on an unfixed cryostat section at the level of the supraoptic nucleus and stained with goat anti-human IgG FITC conjugate. In the four-layer double fluorochrome immunofluorescence test with anti-AVP serum and rhodaminated anti-rabbit serum it was shown that the reacting cells contained vasopressin hormone. Oxytocin cells were unstained. The autoantibodies of this patient also fixed complement.

Demonstration of AVP cell surface antibodies

A pathogenetic effect of AVP cell antibodies is not yet established, but as with thyroid (Khoury et al, 1981a), adrenal (Khoury et al, 1981b) and gastric parietal cell antibodies (Masala et al, 1980) at least some of the cytoplasmic AVP cell antibody-positive sera from patients with idiopathic central DI bind to components of hypothalamic cell membranes. These antibodies are also detected by indirect IFL on cell cultures of human fetal hypothalamus (Scherbaum et al, 1985a). Such antibodies may cause disturbances of AVP cell function either by cytotoxic reactions or by their interference with receptors susceptible to stimulatory or inhibitory inputs.

However, the inhibitory action of AVP cell surface antibodies on the secretion of AVP hormone is still to be demonstrated. The determination of AVP cell surface antibodies is still a difficult task and it will remain restricted to scientific laboratories.

Clinical significance of AVP cell antibodies

Cytoplasmic AVP cell antibodies are detected in one-third of the sera from adult patients with so-called idiopathic central DI (Scherbaum and Bottazzo, 1983) (Table 2). About the same number of sera were positive in cases with onset of DI at the age of one to fifteen years (Scherbaum et al, 1985b). AVP cell antibodies have not been detected in cases of nephrogenic DI or with DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, deafness) syndrome, and they are constantly negative in patients without DI, including those with other hypothalamic disorders or endocrine autoimmune diseases and mixed hospital controls.

Table 2. Prevalence of vasopressin cell antibodies and associated organ-specific autoimmune conditions in 108 patients with different forms of central DI.

Form of DI	Number of cases	Vasopressin cell antibodies (%)	Other organ-specific autoantibodies or diseases	
			Adults (%)	Children (%)
Idiopathic central DI	47	18 (38)	12 (36)	1 (7)
Histiocytosis X with DI	8	5 (63)	0	0
Other forms of central DI	53	1 (2)	5 (16)	0

From Scherbaum et al (1985c), with permission.

The detection of AVP cell antibodies in the serum of a patient is not sufficient to exclude symptomatic DI. More than 50% of the sera from patients with DI due to histiocytosis X contain autoantibodies to AVP cells, whereas the sera from other secondary forms of DI are only positive in occasional cases.

A subgroup of cases with histiocytosis X may now be considered as autoimmune rather than of tumorous origin and this view is supported by several recent findings. In these patients there are thymic abnormalities and antibodies to autologous erythrocytes (Nesbit et al, 1981) and in addition they will respond well to immunosuppressive drug therapy (Osband et al,

1981; Greenberger et al, 1981). The invasion of the hypothalamus by histiocytosis X cells (Cline and Golde, 1973) may lead to a disruption of the blood–brain barrier through a local inflammatory reaction. Histiocytosis X cells bear Class II major histocompatibility molecules on their surface, and by this feature they may be active in stimulating T helper lymphocytes and thus raise an autoimmune response against the surrounding cells. The determination of AVP cell antibodies in cases of histiocytosis X may allow the diagnosis of hypothalamic involvement before the onset of overt central DI. The test may therefore become important for the therapeutic management of histiocytosis X.

DIABETES INSIPIDUS ASSOCIATED WITH ENDOCRINE AUTOIMMUNE DISEASES

The coexistence of an established autoimmune disease with an 'idiopathic' endocrine disorder suggests the possibility of an autoimmune mechanism for the latter. (Nerup, 1974; Schmidt, 1926; Beaven et al, 1959; Carpenter et al, 1964; Neufeld et al, 1980). The first patient in whom AVP cell antibodies were detected had Hashimoto's thyroiditis and Addison's disease, and he then developed 'idiopathic' incomplete central DI. Autoantibodies to adrenal cortex, thyroid microsomes, thyroglobulin and parietal cells were positive in his serum (Scherbaum et al, 1982). A second similar case was described by Bhan and O'Brien (1982).

In a systematic survey of a large number of cases of idiopathic DI it turned out that there is a significant association with endocrine autoimmune diseases. Thyroid autoimmunity is the main association, but also pernicious anaemia, type I diabetes mellitus, primary hypoparathyroidism, alopecia totalis, mucocutaneous candidiasis and others may occur together with idiopathic DI (Scherbaum et al, 1986). In such patients there is a slight female preponderance and the mean age of onset of DI is higher than in the group where no other endocrine abnormality can be observed. This polyendocrine autoimmune syndrome resembles the characteristics of other endocrine autoimmune diseases (Bottazzo and Doniach, 1985).

In a series from a children's hospital, polyendocrine cases were rare; only one out of 13 cases of idiopathic DI had associated endocrine autoimmune diseases, but similar to the cases with onset after childhood, about one-third had vasopressin cell antibodies (Scherbaum et al, 1985b). These features are similar to type I (insulin-dependent) diabetes mellitus (Bottazzo et al, 1981) and they may reflect a different aetiology in the young-onset group.

MORPHOLOGICAL CORRELATES OF CENTRAL DIABETES INSIPIDUS

In DI secondary to infiltration of primary brain tumours or metastatic cells, sarcoidosis (Delaney, 1977), Wegener's granulomatosis (Fauci and Wolf, 1980), histiocytosis X (Tibbs et al, 1978) and others, specific lesions may be

found by biopsy or at post mortem examination (Moses, 1985). However, due to the benign nature of idiopathic central DI, only a few post-mortem examinations of the hypothalamopituitary system have been reported in the literature. In both familial (Braverman et al, 1965; Gaupp, 1941) and non-familial (Blotner, 1958) forms, marked loss of secretory cells in the supraoptic nuclei with localized astrocytic proliferation in that region was described. The paraventricular nuclei likewise showed considerable but less-pronounced cell loss.

In none of the above post-mortem cases were lymphocytic infiltrates detected. Fibrosis and atrophy is also the morphological end-stage of the thyroid gland in primary myxoedema (Simmonds, 1923), the adrenal cortex in Addison's disease (Kracht et al, 1962) and the pancreatic β cells in long-standing cases of type I diabetes (Gepts and Lecompte, 1981), where cellular invasion can be detected within the first six months of diagnosis (Gepts and De Mey, 1978). The lack of lymphocytic infiltrates in idiopathic DI may be due to a burned-out stage of the disease which had lasted for 14–32 years as documented in the above-described cases. Clinical pathologists should be aware that they can add a valuable piece of information to our present knowledge of hypothalamic autoimmunity in cases of idiopathic DI of recent onset. Most likely, lymphocytic infiltration will be observed in these recent cases if they come to the histological examination.

Animal models of inherited diabetes insipidus

The Brattleboro (B) rat is the animal model of hereditary DI that has been most extensively studied (Valtin, 1982). Homozygote B rats do not produce vasopressin and AVP is markedly reduced or absent in the posterior pituitary (Russell et al, 1980). A single nucleotide deletion in the protein-coding region of the AVP precursor seems to be responsible for a wrong reading sequence and AVP-messenger RNA translation (Schmale and Richter, 1984). It has been demonstrated, however, that AVP-like molecules may be synthesized in ovary (Lim et al, 1984) adrenal (Nussey et al, 1984), sympathetic ganglia (Hanley et al, 1984) and testis (Kasson et al, 1985). It was also demonstrated by cell culture studies that immunoreactive AVP is contained within and released by anterior pituitary cells (Lolait et al, 1986). As AVP can potentiate or act synergistically with the corticotropin releasing factor at the anterior pituitary a paracrine or autocrine action for AVP is suggested in these tissues.

Animal models of polydipsic disorders and nephrogenic DI have also been valuable tools for the understanding of the corresponding disease in humans (Valtin et al, 1985).

Experimental autoimmune hypothalamitis

To satisfy Witebsky's criteria for the diagnosis of autoimmune diseases, an animal experiment should be available to prove the autoimmune origin of hypothalamitis at least in some cases of human central DI. Preliminary experiments have been performed where guinea pigs were injected with

homogenized tissue from the area of the supraoptic nucleus together with complete Freund's adjuvant, and repeatedly boosted with the antigen (W. Scherbaum, unpublished experiments). These studies were complicated by the induction of experimental allergic encephalomyelitis (Lassmann et al, 1980; Holoshitz et al, 1983), most likely through the presence of contaminating basic myelin protein in the extracts. New experiments are under way where animals are neonatally tolerized (Teale and Klinman, 1980; Luzuy et al, 1986) to non-hypothalamic brain extracts and later injected with the specific antigens. Such trials, however, are very tedious and the results are not clearly predictable since there is a large number of different antigens present in the supraoptic area.

CONCLUSIONS

Hypothalamic autoimmunity is a fairly recent acquisition in the growing spectrum of endocrine autoimmunity. Technical problems still exist to finally establishing this new pathological entity. The availability of reliable tissue substrate remains a major obstacle in further developing this subject. Tissue culture techniques have been improved and there is hope that by taking advantage of the more advanced, newly accumulated and more common endocrine autoimmune diseases we shall reach the stage of devising new experimental systems and opening insights into the still obscure area which involves several hypothalamic conditions.

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