AUTOANTIBODIES TO VASOPRESSIN CELLS IN IDIOPATHIC DIABETES INSIPIDUS: EVIDENCE FOR AN AUTOIMMUNE VARIANT

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Summary
Autoantibodies to vasopressin-secreting cells of human hypothalamus were detected by means of indirect immunofluorescence (IFL) in 13 patients with diabetes insipidus (DI). 11 of 30 patients (36-7%) with "idiopathic" and 2 of 32 (6-3%) with symptomatic DI were positive, and 139 control patients were negative. The specificity of the reaction to vasopressin cells was demonstrated with a 4-layer double-fluorochrome IFL test in which the second sandwich consisted of rabbit anti-vasopressin or anti-oxytocin counterstained with rhodaminated anti-rabbit immunoglobulin. 5 patients had also antibodies to oxytocin-producing cells. The antibodies reacted with cytoplasmic components distinct from the hormone; they were of IgG, IgA, or IgM class or a combination of these classes, and half of them fixed complement. Maximum titres were 1:32, and the antibodies could not be absorbed out by incubation with vasopressin, oxytocin, neurophysin I, or neurophysin II. Some sera stained as yet unidentified small cells in the hypothalamus. This report suggests that autoimmunity extends to the hypothalamus. Vasopressin-cell antibodies may prove to be useful markers for the diagnosis of an autoimmune variant of diabetes insipidus.

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REFERENCES—continued

**Introduction**

DIABETES insipidus (DI) is a syndrome characterised by polyuria and polydipsia, and in the central (vasopressin-sensitive) form symptoms are due to a lack of arginine vasopressin (AVP), a hormone produced in the supraoptic and paraventricular nuclei of the hypothalamus. Ischaemic lesions, haemorrhage, cellular infiltration with disseminated or localised tumours or granulomas, and traumatic destruction of these centres may lead to permanent DI. However, the cause of 30–50% of cases remains unknown. Some cases are familial with onset of symptoms soon after birth, but idiopathic DI is usually sporadic and can start at any age.

Post-mortem examination of the hypothalami has been reported in only a few cases of familial and non-familial idiopathic central DI. Each showed a heavy loss of secretory cells and severe gliosis of the supraoptic nuclei and similar but moderate changes in the paraventricular nuclei. All these patients had had the disease for many years, and no lymphoid infiltration was observed.

Since the first detection of thyroid autoantibodies in Hashimoto’s thyroiditis, autoimmune variant has been recognized in most endocrine disorders previously thought to be idiopathic, and in some patients more than one endocrine gland is involved. Idiopathic DI is unexpectedly often associated with autoimmune diseases or with organ-specific autoantibodies (Scherbaum WA, et al., unpublished). This prompted us to look for autoantibodies to hypothalamic vasopressin-secreting cells in DI patients. Our findings provide the first evidence for a possible autoimmune variant of diabetes insipidus.

**Patients and Methods**

Clinical data were available for most of the patients studied, and the diagnosis of DI had been established by recognised criteria. All 62 patients had been treated with various types of hormone replacement therapy before serum was collected for antibody tests. The clinical characteristics of patients with DI are summarised in the table. The 9 cases with associated organ-specific autoimmune diseases included autoimmune thyroiditis, Addison’s disease, type 1 diabetes, pernicious anaemia, primary hypoparathyroidism, Sjögren’s syndrome, myasthenia gravis, and primary amenorrhoea. 7 patients had more than one of these disorders associated with DI.

2 patients had significant titres of thyroid and islet-cell antibodies in the absence of overt thyroid or early pancreatic abnormalities. The 26 patients with symptomatic DI included patients with histiocytosis X (3), persistent postoperative DI (10 pituitary adenomas and 2 craniohypophysialomas), transient postoperative DI (3 pituitary adenomas, 1 craniohypophysialoma), post-pituitary irradiation (1), metastatic tumours (3), acute myeloid leukaemia (1), sarcoidosis (1), and post-traumatic DI (1). 5 of these patients had low titres of thyroid or parietal-cell antibodies. 6 further patients had symptomatic DI due to DIDMOAD syndrome (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness). All the sera were kept at −20°C until tested.

**Controls**

The 139 control subjects did not have DI, and they included patients with hypothyroidal diseases (7), mixed pituitary disorders (5), and polyendocrine autoimmune abnormalities (31) and mixed hospital controls (76).

**Tests for Conventional Autoantibodies**

Antibodies to gastric parietal cells, pancreatic islet cells, adrenal cortex, and steroid-producing gonadal cells were measured with the standard indirect immunofluorescence (IFL) technique on unfixed cryostat sections of human organs obtained from donors with blood group O. Thyroid microsomal and thyroglobulin antibodies were tested by means of passive haemagglutination with commercial kits (Wellcome, London).

**Hypothalamic Tissue Substrates**

5 human hypothalami from adults (aged 38–91 years) were obtained at necropsy 2/4 to 6/8 hr after death. 6 fetal hypothalami were obtained from therapeutic hysterotomies between 18 and 22 weeks’ gestation. They were cooled immediately to 4°C and snap-frozen within 1–4 h. Fresh baboon hypothalamus was snap-frozen immediately after dissection. All tissues were kept at −70°C until used.

Serial cryostat sections were cut in the coronal plane starting from the optic chiasma through to the mamillary bodies. Before the IFL tests, the areas with large secretory cells and typical granular cytoplasmic content were localised with gelsma and haematoxylin and eosin staining of different sections cut through the block containing the supraoptic and paraventricular nuclei. The area of the paraventricular nucleus also contained compartments of smaller and less granulated cells.

**Hormone Antiserum**

The presence of vasopressin-secreting and oxytocin-secreting cells on the cryostat sections of the supraoptic and paraventricular nuclei was confirmed by indirect IFL using specific rabbit anti-AVP and anti-oxytocin sera (kindly provided by Prof. J. S. Jenkins, Dr J. Hawthorn, and Profesor Dierickx, respectively). Anti-AVP was used at dilution 1/10 and anti-oxytocin at 1/5 in all the experiments described. Purification procedures of anti-AVP sera by means of affinity chromatography immunoabsorption with oxytocin reduced the strength of the antiserum to such an extent that it was not suitable for the 4-layer double-fluorochrome IFL test. The same applied to oxytocin antiserum when it was cross-absorbed with AVP.

**Immunofluorescence Tests**

Patients’ undiluted sera were incubated on unfixed cryostat sections for 40 min at room temperature, then washed in phosphate-buffered saline, pH 7.4. Antibodies were stained with fluorescein isothiocyanate (FITC)-conjugated goat anti-human F(ab)2. The Ig

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**TABLE 1: CLINICAL CHARACTERISTICS OF PATIENTS WITH CENTRAL DIABETES INSIPIDUS**

<table>
<thead>
<tr>
<th>Types of DI</th>
<th>No. of patients</th>
<th>Sex ratio</th>
<th>Mean age (range)</th>
<th>Mean age at onset of DI</th>
<th>No. of patients with Associated autoimmune diseases</th>
<th>No. of patients with Organ-specific autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic DI</td>
<td>30</td>
<td>16M/14F</td>
<td>34-9 yr (10-73 yr)</td>
<td>26-6 yr (2-59 yr)</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>DIDMOAD syndrome</td>
<td>6</td>
<td>2M/4F</td>
<td>42-4 yr (1 mo-72 yr)</td>
<td>At birth</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other forms of symptomatic DI</td>
<td>26</td>
<td>11M/15F</td>
<td>36-9 yr (1 mo-71 yr)</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

DI = diabetes insipidus.
DIDMOAD = Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness.
class was established with specific anti-IgG, anti-IgA, and anti-IgM conjugates. The complement-fixing ability of the patients' antibodies was studied after addition of fresh normal human serum and an anti-human C3, FITC-conjugate. To identify the reactive cells, the 4-layer-double-fluorochrome IFL test was done on acetone-fixed sections as previously described. On ultraviolet microscopy the technique shows red and green staining on separate or identical cells according to whether or not the patients' antibodies and the rabbit anti-hormone sera react with the same or different cells. The identity of the reaction to the same cells was confirmed by double-exposure colour photography.

**Absorption Experiments**

600 μl samples of serum from a patient positive for antibodies to large secretory hypothalamic cells at titre 1:16 were absorbed separately with 200 μl mixtures containing 10 IU oxytocin, 10 IU AVP, 0.4 mg human neurophysin I, and 0.2 mg human neurophysin II. As controls, rabbit anti-AVP (titre 1:10), anti-oxytocin (titre 1:5), anti-neurophysin I (titre 1:40), and anti-neurophysin II (titre 1:20) were absorbed with half the amount of the corresponding hormones listed above. The mixtures were incubated for 30 min in a 37°C water bath and then shaken overnight at 4°C. After centrifugation at 3000 rpm for 10 min, the absorbed sera were tested with IFL on unfixed cryostat sections of human and baboon hypothalami.

**Results**

**Selection of Optimum Tissue Substrates**

Fresh tissue proved to be essential for the detection of hypothalamic antibodies, and human fetal material was found to provide a better substrate than adult hypothalami. Only 4 of 13 sera which were positive for AVP-cell antibodies on several substrates reacted on adult brain obtained 3 h after death. The age of the donor was also crucial. Specimens obtained from subjects older than 50 years were unsuitable because of an accumulation of lipofuscin in the secretory cells, which gave an orange autofluorescence and thus interfered with the reading of the specific IFL. Antibody results obtained on fresh tissue from young baboons were comparable with those on human fetal hypothalamus (see figure).

**Antibodies to Vasopressin Cells**

Table II shows the overall prevalence of hypothalamic autoantibodies in patients with idiopathic and symptomatic DI compared with patients with hypothalamic, pituitary and polycordocrine disorders and mixed hospital controls. 11 (37%) of the 30 patients with idiopathic DI had antibodies to vasopressin cells. The same specificity was also detected in 2 patients with symptomatic DI, both of whom had histiocytosis X.

The precise characterisation of the patients' hypothalamic autoantibodies was obtained with the 4-layer-double-fluorochrome IFL test, in which the rabbit anti-AVP serum stained the same cells as the patients' serum. 5 AVP-cell-positive sera also reacted with cells recognised by rabbit anti-oxytocin serum.

**Table II.--Prevalence of Autoantibodies to Vasopressin (AVP) and Oxytocin (Oxy) Cells of Human Hypothalamus in Patients with Hypothalamic and Other Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients tested</th>
<th>AVP and/or Oxy cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic central DI</td>
<td>30</td>
<td>11 (72%)</td>
</tr>
<tr>
<td>DIDMOAD syndrome</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Other forms of symptomatic DI</td>
<td>26</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Hypothalamic diseases without DI</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Mixed pituitary disorders</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Polyendocrine autoimmune disease</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Mixed hospital controls</td>
<td>76</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>201</td>
<td>13 (6%)</td>
</tr>
</tbody>
</table>

Table III summarises the clinical and immunological characteristics of the patients positive for AVP-cell antibodies. In the group with idiopathic DI there was a preponderance of males (7M/4F), and the mean age was 26 years. 7 of these patients (63%) had one or more associated endocrine autoimmune diseases and/or organ-specific autoantibodies. AVP-cell antibodies were predominantly of IgG and IgA classes (5 sera had both), and only one serum was positive for IgM antibodies. Antibody titres ranged from 1:1 to 1:32, and half the positive sera also fixed complement (table II).

**Antibodies to Unidentified Small Hypothalamic Cells**

15 sera reacted with cells that were not stained with anti-AVP or anti-oxytocin sera. These separate autoantibodies were seen in a substantial proportion of patients with idiopathic central DI, but they were also detectable in a small proportion of other patients studied (table II).

**Absorption Studies**

Absorption of one of the sera positive for AVP-cell antibodies with an excess of synthetic AVP, oxytocin, and human neurophysin I and II did not affect the IFL reactivity, whereas half the amount of these hormones was sufficient to abolish the specific staining of hypothalamic cells by the corresponding hormone antisera.

**Discussion**

Central diabetes insipidus (DI) is a rare disorder and consists of a heterogeneous group of underlying diseases, some of which are detectable only at necropsy. Most of the spontaneously acquired cases are diagnosed as "idiopathic DI", and the demonstration of autoantibodies to AVP-secreting cells in 37% of such patients suggests that there
TABLE III—CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS OF PATIENTS WITH AUTOANTIBODIES TO AVP AND OXYTOCIN CELLS

<table>
<thead>
<tr>
<th>Case no</th>
<th>Sex/age</th>
<th>Diagnosis</th>
<th>Age of onset (yr)</th>
<th>Associated diseases</th>
<th>Age of onset (yr)</th>
<th>Hypothalamic antibodies</th>
<th>Other cells</th>
<th>Other antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/22</td>
<td>Complete idiopathic DI</td>
<td>14</td>
<td>Alopecia totalis, paraneuronal encephalitis</td>
<td>8</td>
<td>IgG - IgM - IgA - C3</td>
<td></td>
<td>Adrenal cortex, gonadal steroid cells, pituitary pituitary cells, intrinsic factor</td>
</tr>
<tr>
<td>2</td>
<td>F/24</td>
<td>Primary hyperparathyroidism</td>
<td>10</td>
<td>Primary hyperparathyroidia, primary hyperparathyroidia</td>
<td>19</td>
<td>2 - - - -</td>
<td></td>
<td>Thyroid</td>
</tr>
<tr>
<td>3</td>
<td>M/61</td>
<td>Primary hyperparathyroidism</td>
<td>59</td>
<td>Primary hyperparathyroidia, primary hyperparathyroidia</td>
<td>58</td>
<td>2 - - - -</td>
<td></td>
<td>Thyroid</td>
</tr>
<tr>
<td>4</td>
<td>M/21</td>
<td>Sjögren's syndrome</td>
<td>16</td>
<td>Sjögren's syndrome</td>
<td>3</td>
<td>1 - 4 - -</td>
<td>+</td>
<td>Salivary ducts</td>
</tr>
<tr>
<td>5</td>
<td>F/10</td>
<td>Diabetes mellitus</td>
<td>2</td>
<td>Diabetes mellitus</td>
<td>6</td>
<td>- - 2 -</td>
<td>-</td>
<td>Islet cells, gastric parietal cells</td>
</tr>
<tr>
<td>6</td>
<td>M/33</td>
<td>Diabetes mellitus</td>
<td>28</td>
<td>Diabetes mellitus</td>
<td>-</td>
<td>4 - 2 - 1</td>
<td>+</td>
<td>Thyroid</td>
</tr>
<tr>
<td>7</td>
<td>M/14</td>
<td>Diabetes mellitus</td>
<td>11</td>
<td>Diabetes mellitus</td>
<td>-</td>
<td>- - 2 - 8</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>F/38</td>
<td>Diabetes mellitus</td>
<td>38</td>
<td>Diabetes mellitus</td>
<td>-</td>
<td>- - 4 - 4</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>F/47</td>
<td>Partial idiopathic DI</td>
<td>44</td>
<td>Partial idiopathic DI</td>
<td>38</td>
<td>8 - 16 - 8</td>
<td>-</td>
<td>Thyroid, gastric parietal cells, adrenal cortex, gonadal steroid cells, rheumatoid factor</td>
</tr>
<tr>
<td>10</td>
<td>M/53</td>
<td>Partial idiopathic DI</td>
<td>44</td>
<td>Partial idiopathic DI</td>
<td>38</td>
<td>8 - 16 - 8</td>
<td>-</td>
<td>Thyroid, gastric parietal cells, adrenal cortex, gonadal steroid cells, rheumatoid factor</td>
</tr>
<tr>
<td>11</td>
<td>M/21</td>
<td>Juvenile melanoma</td>
<td>2</td>
<td>Juvenile melanoma</td>
<td>8</td>
<td>1 - - 2</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>F/17</td>
<td>Histiocytosis X</td>
<td>14</td>
<td>Histiocytosis X</td>
<td>15</td>
<td>- - 1 - 4</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>M/23</td>
<td>Histiocytosis X</td>
<td>19</td>
<td>Histiocytosis X</td>
<td>18</td>
<td>- - 1 - 2</td>
<td>-</td>
<td>None</td>
</tr>
</tbody>
</table>

exists a previously unrecognised autoimmune variety of this disorder.

The choice of tissue was crucial for the detection of AVP-cell antibodies. The brain cools slowly after death, and, in contrast to the well-preserved neurotransmitters and hormones, cytoplasmic autoantigens accounting for the specific AVP-cell staining are rapidly inactivated, so that tissues obtained more than 4 hr after death were unsuitable. This is probably why we found twice as many positive cases when fresh human fetal hypothalamus was used. Another difficulty with adult necropsy tissue arises from the natural accumulation of lipofuscin granules in secretory hypothalamic cells, especially in older donors. This produces an orange autofluorescent, which interferes with the reading of the specific IFL. Preliminary results obtained with fresh monkey hypothalami from young animals indicate that this substrate gives good cross-reactivity with the human antibodies, and it may be a suitable material for testing hypothalamic antibodies in routine screening procedures in the future.

The reactivity of AVP-cell antibodies was not affected by preabsorption of the positive sera with AVP, oxytocin, or their corresponding neurophysins; thus the IFL staining originally observed was not the result of passive immunization by replacement therapy with various hormone preparations. Therefore, antibodies to AVP cells in DI patients do not react with the hormone itself, but they probably recognize membrane-bound cytoplasmic autoantigens equivalent to the known antigen-antibody systems of the thyroid gland, pancreatic islet cells, and adrenal cortex.

About 60% of patients with antibodies to AVP cells also have signs of other autoimmune endocrine abnormalities, which suggests that polyendocrine autoimmunity extends to the hypothalamus. This conclusion is also supported by the fact that a third of the patients with idiopathic central DI have associated autoimmune endocrine disorders regardless of the presence of detectable hypothalamic antibodies.

The absence of AVP-cell antibodies in two-thirds of the patients with idiopathic DI may in some cases be due to non-autoimmune damage to the hormone-producing cells. However, in the majority of our patients the onset of DI antedated investigation of serum by many years, and endocrine autoantibodies tend to disappear over the years, especially in juvenile type-I (insulin-dependent) diabetes mellitus. Only by testing newly diagnosed patients can the real prevalence of AVP-cell antibodies in DI be established, and regular follow-up studies of such patients will help to define more precisely the heterogeneity of diabetes insipidus.

AVP-cell antibodies seem to occur preferentially in patients with idiopathic central DI, but their demonstration in 2 patients with histiocytosis X suggests that immunological events may also be involved in some cases of symptomatic DI. A pathogenic effect of AVP-cell antibodies is not yet established; but, as with thyroid, adrenal, and gastric antibodies, there might exist surface-binding antibodies to AVP-cells which may cause cytotoxic reactions or interfere with receptors susceptible to stimulatory or inhibitory inputs.

The demonstration of autoimmune reactions in central diabetes insipidus may stimulate investigation of other hypothalamic diseases so far considered "idiopathic." Why,
SYMPATHETIC NEURAL PROSTHESIS FOR MANAGING ORTHOSTATIC HYPOTENSION

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Summary

A prototype electromechanical analogue of the sympathetic division of the baroreceptor reflex arc was used to maintain blood pressure automatically in two patients with neurogenic orthostatic hypotension. The device prevented significant and sustained reductions in mean blood pressure when the patients were tilted up to 85°. Upon achieving the preset mean blood pressure, the device maintained this pressure with a standard error of less than 2 mm Hg. Similar results were obtained when the patients were walking. The device did not cause supine hypertension during the trials.

Introduction

ORTHOSTATIC hypotension is probably the most disabling symptom of chronic autonomic failure. Patients lack the normal compensatory adjustments to blood pressure that occur upon standing or in response to the Valsalva manoeuvre, digestion, or other stimuli which cause vasodilatation.1,2 Little progress has been made towards improved therapy; some success has been reported with several drugs,3-6 but undesirable side-effects, including supine hypertension, limit their usefulness.7 Atrial tachypacing has also been used but the inability of a demand pacemaker to modulate blood pressure adequately in response to functional requirements is a major disadvantage.

Maintenance of normal blood pressure while standing is accompanied by a doubling of the plasma noradrenaline level.8 In patients with neurogenic orthostatic hypotension the plasma noradrenaline level does not rise appropriately upon standing;9 they have greater than normalpressor responses to intravenous noradrenaline,10 which can maintain blood pressure and alleviate symptoms while standing (unpublished).

We report the development and initial clinical testing of a prototype device which emulates the physiological responses of the baroreceptor reflex arc. The patient’s blood pressure is maintained automatically within a specified range despite changing conditions and demands of the body.

Patients and Methods

Patients

Two patients with multiple system atrophy were selected for this study; patient 1 was a 33-year-old woman and patient 2 a 42-year-old man, the two youngest patients in our autonomic research programme. They were chosen because they were in good general health and appeared subject to the fewest risks. Patient 1’s earliest symptom (postural dizziness) appeared at age 30. Impotence was the heralding sign at age 36 in patient 2. In both patients there was evidence of panautonomic including orthostatic hypotension, neurogenic bladder, severe constipation, abnormal pupillary function, and lower than normal sweating. Abnormal Valsalva responses, reduced sinus arrhythmia, normal baseline plasma noradrenaline levels with inadequate postural increments, and exaggeratedpressor responsiveness to administered noradrenaline and angiotensin II were demonstrated, as in previous patients with multiple system atrophy.10,11 Neurological findings included

References