AGONISTIC AUTOANTIBODIES, A RISK FACTOR IN PATIENTS WITH TYPE 2 DIABETES

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ABSTRACT — In addition to insulin intolerance, patients with type 2 diabetes suffer from hypertension, renal insufficiency, retinopathy, wound healing disorders, coronary heart disease, heart attacks, strokes, and amputations. In addition to metabolic syndrome, many patients have pathological changes in macro- and microcirculation. One of the causes might be agonistic autoantibodies (agAAB), an immunological component. This specialized group of autoantibodies activates the G protein-coupled receptors similar to the way natural agonists do and triggers receptor-specific reactions in the cell (1). The pathological potential of agAAB has been described in numerous publications. The pathological processes triggered by agAAB for the ß-1-adrenoceptors (AR), AT1 AR, and α 1 AR (2,3,4,5) have been particularly well researched. Animal experiments provided valuable insights into the causality of receptor-specific autoantibodies for the development of diseases and disease-relevant symptoms. These autoantibodies can only be removed with specific antagonists at the receptor or by plasmapheresis or immunoadsorption. The agAAB do not respond to immunosuppression as classical autoantibodies do. Patients in whom agAAB was removed by extracorporeal treatment benefited from it. In patients with dilated cardiomyopathy, cardiac output improved (6,7); those with Alzheimer's disease (8) achieved stabilization of cognition. In subjects with Thromboangiitis obliterans (9), further amputations were able to be avoided after removal of the autoantibodies, and in patients with inadequate control of hypertension through pharmacological means, blood pressure was considerably reduced (10). In only a few cases did agAAB reappear. These positive treatment results for various diseases formed the basis for screening diabetics with respect to the prevalence of agonistic autoantibodies.

INTRODUCTION

There are currently 425 million people worldwide who have been diagnosed with type 2 diabetes. A further 179 million people already have this disease, but are not yet aware of it. The WHO assumes that by 2045, a total of 700 million people worldwide will be suffering from type 2 diabetes and associated health impairments. In Germany alone, the number of new cases is 442,000 people annually, or more than 1,000 people per day. Untreated, this disease has dramatic consequences. Macro- and microangiopathies have been diagnosed in patients, which lead to terminal organ damage if left untreated. Diabetics more frequently suffer a heart attack or stroke. They suffer more often from congestive heart failure and dementia than non-diabetics. The risk of myocardial infarction in post-menopausal women is six times higher among diabetics than for non-diabetics (Table 1). The risk of developing dementia by diabetics is twice as high as for people without diabetes [11, 12, 13].

The number of amputations in Germany is 40,000 patients per year, 2000 patients go blind, and 30–40% of diabetics experience kidney damage. Some of them require dialysis. Nephropathy is promoted by poorly regulated glucose levels and by blood pressure levels above 120–130/70–80 mm Hg. The treatment costs for patients with type 2 diabetes are enormous. The pharmaceutical industry expects its sales of medications for diabetics will increase by 50% between 2016 and 2022. In order to counteract a further increase in cases of disease and the considerable associated costs, possible additional causes that influence the genesis of this disease must be sought. One possible additional risk factor might be agonistic autoantibodies that act against various G-protein-coupled receptors [14]. AgAAB against the ß-1 AR, ß-2 AR, endothelin receptor, angiotensin II type-1 receptor, and α-1 AR were detected in sera of 150 diabetics. It was found that within the first five years following diagnosis of diabetes type 2, 48% of the test subjects had at least one agonistic autoantibody, five years later the prevalence was 66%, and after more than 20 years the number increased to 68%. Agonistic autoantibodies against the α-1 receptor were dominant in all the patient groups studied. Their prevalence increased from 69.6% to 81.8% for both positively detected subjects.

Agonistic autoantibodies acting against α-1 AR have considerable pathological potential. Binding of agAAB to the a-1 AR leads to activation of the receptor, similar to the action of physiological agonists. The increase of intracellular Ca^{2+} transient means acute elevation of intracellular Ca^{2+}, hypertrophic remodeling due to this increased intracellular Ca^{2+}, phosphorylation of cardiac regulatory proteins and other phosphorylation of target proteins (i.e. the 15-kDa) protein phospholemman — a cardiac regulator of $Na+/Ca^{2+}$ exchanger and Na^{+}/K^{+} ATPase), activation of protein kinase C, and proliferation of vascular smooth muscle

Table 1.

	Infarct	CHD	Apoplexy	CHF	Atrial fibrillation	Cancer
Diabetics	9.0	15.8	6,4	6.9	8.8	10.5
Non-diabetics	4.3	7.9	3,9	3.3	5.6	9.9

cells. It also leads to hyperplasia, to triggering of various pathological mechanisms by activation of the receptor, causes a reduction of the vessel lumen in the vessels, and the increase in calcium transient decreases the calcium concentration in the mitochondria and endoplasmic reticulum [15, 16, 17, 18].

METHODS/MATERIAL

An ELISA test developed in-house was used for the detection of agonistic autoantibodies. Autoantibody analysis was performed using peptides corresponding to the first and/or second extracellular loops of the following GPCR: α-1 AR, endothelin A, angiotensin II type-1 AR, ß-1 AR, ß-2 AR and protease-activated receptor (PAR) 1/2. Peptides were coupled to pre-blocked streptavidin-coated 96-well plates (Perbio Science, Bonn, Germany). Patient serum was added in a 1:100 dilution and incubated for 60 min. A horseradish peroxidase conjugated anti-human IgG antibody was used as the detection antibody (Rockland Biomol GmbH, Hamburg, Germany). Antibody binding was detected by the 1-Step Ultra TMB ELISA (Perbio Science, Bonn, Germany). The absorbance was measured at 450 nm against 650 nm with a SLT Spectra multiplate reader (TECAN, Crailsheim, Germany)

OUTCOMES

3 patient groups of 50 subjects each were examined:

Group 1: diabetes duration 0–5 years, prevalence 48%

Group 2: diabetes duration 6–10 years, prevalence 66%

Group3: subjects /cardiovascular complications prevalence 46% (heart attack/stroke/stents),

In Group 3, the largest group (n= 31) had a stent.

Of these patients, 14 had an agAAB against the α-1AR.

Of the 18 subjects with myocardial infarction, 8 had a positive result with respect to α-1 AR.

In subjects testing positive for agAABs, the distribution of the various agAABs is shown in Table 2.

It was notable that 66% of the test persons in Group 1 and 76% in Group 2 had systolic blood pressure values above 130 mm Hg several antihypertensive medication revenue. Diastolic blood pressure was over 80mm Hg in 72% of patients. These blood pressure levels promote the development of diabetic nephropathy. As kidney damage progresses, the structure of the filtering domains is increasingly destroyed, creating actual holes in the renal corpuscles [19, 20].

In an animal experiment with male Wistar rates (10–13 weeks of age; 280–350g) we were able to show the effect of agonistic agAAB on the kidneys of the animals. Two experimental cohorts 10 rats each were allocated at random. One cohort of animals (PEP) was immunized by subcutaneous injection of 300 µg α-1-AR peptide coupled to BSA and emulsified in incomplete Freund's adjuvance at 0, 2 and 4 weeks. Then the injections were repeated monthly for 8 month. The respective control animals (C-PEP) were subcutaneously injected with BSA. Blood aliquots were taken from anesthetized animals by retro-orbital sampling. The obtained sera were analyzed for the presence of α-1AR antibodies by ELISA techniques. The rates we obtain from Charles River Laboratories, Sulzfeld, Germany.

In rats positive for agAAB against α-1 AR (Fig.1B), we were able to observe a change in kidney tissue after 8 months with immunohistochemistry with CD31 in contrast to the control animals (Fig.1A). The animals also developed holes in the glomerular filter without suffering from diabetes. As a result, ever larger quantities of protein are lost.

An animal experiment by a Chinese research group has shown that the holes in the kidney in diabetic rats are first formed by agAAB against the α1-AR [21].

Table 2.

Receptor	α 1 AR	ß-2 AR	ß-1 AR	AT-1	ETA
Group 1	69.6%	56%	52%	39%	39%
Group 2	81.8 %	75.8%	63.6%	21%	42%
Group 3	60,8%	52,1%	43.5%	17%	17%

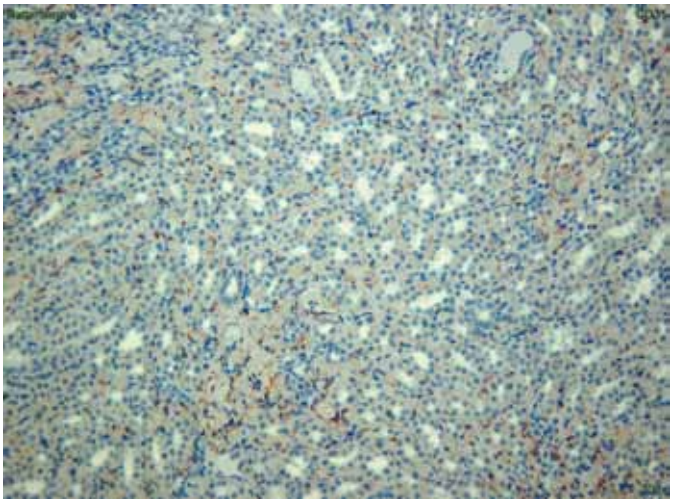

Fig. 1A. Control animal untreate 8 months old without diabetes

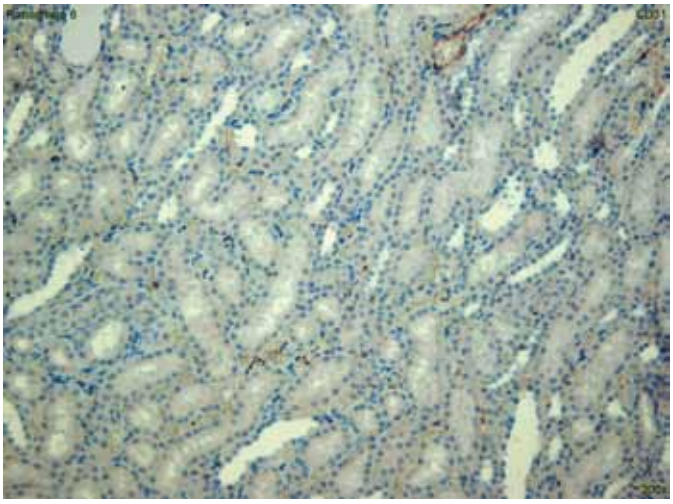

Fig. 1B. Changes after eight months in a 1-AR positive animal without diabetes

CONCLUSIONS

Agonistically acting autoantibodies represent an additional risk factor for patients with diabetes type 2 due to the pathological mechanisms triggered by them. The activation of the α-1 adrenergic receptor by agAAB activates calcium homeostasis. An increase of cytosolic Ca^{2+} represents a threatening development for the cell and leads to irreversible damage to it — up to and including cell death — if not buffered and eliminated. Free and protein-bound Ca^{2+} ions trigger intracellular signaling cascades, such as the activation of calcium-dependent cell death proteases (calpains), and thus act as secondary messengers for the cytotoxicity that occurs. This cell loss leads to, among other things, a change in the morphology of the renal cell tissue with the consequence of reduced filtration capacity in the kidney. The resulting renal insufficiency leads to compulsory dialysis in many patients.

Protein kinase C (PKC) requires cellular calcium for its correct functioning. Calcium activates hypertrophic remodeling, i.e. the vascular wall thickens inwards (18). Due to the long duration of the agAAB binding to the receptor, activations take place over a long period of time (7–21 days). As a result, normal cell state is not attained and therefore the pathological parameters are expanded. Protein kinase C plays a central role in signal transduction (10). Its activity is controlled by hormones and neurotransmitters whose signals are transmitted via secondary messengers. Calcium is required for the functioning of PKC. Calcium is released from the endoplasmic reticulum and/or from the mitochondria. ATP and proteins serve as substrates. Permanent receptor activation leads to overload of the cell with calcium. PKC is important for the regulation of cellular growth. Malfunction can be involved in triggering cancer and in the development of late complications in diabetes. Short-term changes in the intracellular concentration of cytosolic calcium concentrations can be compensated for by control mechanisms. However, if there is a pronounced change in equilibrium or if transport processes are chronically disturbed (for example by the reduction of the Ca^{2+}-ATPase or by perturbed Na^{+}-Ca^{2+} exchange), the cell becomes overloaded with calcium ions and cytosolic calcium concentration increases chronically [14]. This leads to activation of messenger systems such as calmodulin and protein kinase C. These changes lead to chronic changes in the cell or even to cell death. The permanent activation of the signal cascades leads to pathological cell changes, as the cell no longer returns to its normal resting state. Na^{+}/K^{+} ATPase [16] regulates the transport of Na^{+} from the cell and the transport of K^{+} into the cell. ATP is hydrolyzed to ADP. Dysregulation or lack of neuronal Na^{+}/K^{+}-ATPase can lead to neuronal dysfunction and behavioral abnormalities. Moreover, neurodegeneration can be triggered.

In all the subject groups studied, it was conspicuous that autoantibodies against the ß-2 AR were often the most frequently detectable after autoantibodies against α-1 AR. AgAAB against ß-2 AR also activate this receptor in a non-physiological manner. ß-2 AR activation couples to the adenylate cyclase system, leading to increased cAMP formation and activation of protein kinase cascades that influence numerous processes such as glycogenolysis, cellular calcium flows,

immune responses, storage and learning processes, as well as gene expression. AgAAB against ß-2 AR may therefore cause dysregulation of the adenylate cyclase / cyclic AMP system and may lead to abnormalities in energy metabolism and neuronal function, for example. As shown by Ni et al., the activation of ß2 AR by the selective agonist clenbuterol stimulates γ-secretase and increases the production of amyloid ß40 and ß42 [22]. It must be assumed that agAAB also has that effect against the ß-2 AR and, like an agonist, triggers amyloid ß production by stimulating the receptor. This activation of the signal cascades as described might be a possible cause for diabetics developing dementia more frequently than people without diabetes [23, 24, 25]. Autoantibodies against the endothelin-A receptor also act like natural agonists and are thus involved in increased vasoconstriction. Only autoantibodies against the ETA receptor were sought in the present prevalence study. Angiotensin II causes vasoconstriction in blood vessels and an increased release of aldosterone in the adrenal cortex.

The processes described, triggered due to activation of adrenoceptors by agAAB as well, and considerable prevalence of agAABs in patients with diabetes type 2, should be given greater attention in the treatment of diabetics in future. It is possible that a number of secondary diseases could be greatly reduced or avoided by early pharmacological intervention or by immunoadsorption. Prerequisite for treatment is the diagnosis of agAAB.

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CONFLICT OF INTEREST STATEMENT

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Animal experiments were carried out in accordance with the guidelines provided and approved by the animal welfare department of the Landesamt für Gesundheit und Soziales Berlin (Berlin State Office of Health and Social Affairs, Permit Number: G0197/10).

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