Crossreactivity of antibodies to Neisseria gonorrhoeae with Hsp60 correlates with reduced mitochondrial activity in the choroid plexus papilloma cell line HIBCPP

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Introduction

First trimester maternal infections with the Gram-negative bacterium Neisseria gonorrhoeae (NG), increase the risk for the offspring to suffer from psychotic symptoms later in life.* We hypothesize here an immune- mediated mechanism for this, and investigated therefore interactions of a rabbit antiserum directed to NG (α-NG) with postmortem samples of human choroid plexus and with HIBCPP cells, an *in vitro* model of human choroid plexus epithelium.

* Sørensen et al., 2009, Schizophrenia Bull. 35, 631-637.

Materials&Methods

Antibodies and proteins: Anti-Neisseria gonorrhoeae (**α-NG**): rabbit polyclonal, Antikoerper-online.de, Cat. Nr. ABIN285584. Anti-Neisseria meningitidis (**α-NM**): rabbit polyclonal, Antikoerper-online.de, Cat. Nr. ABIN285585. **Anti-rabbit IgG,** goat polyclonal, Peroxidase coupled, Sigma-Aldrich, Cat. Nr. A9169. **Anti-rabbit IgG**, goat polyclonal, Biotin coupled, Sigma-Aldrich, Cat. Nr. B8895. Recombinant human heat shock protein **Hsp60**, Antikoerper-online.de, Cat. Nr. ABIN621577. Recombinant human ATP-Binding Protein **(ATPB)**, Antikoerper-online.de, Cat. Nr. ABIN1346052.

Cell culture: HIBCPP cells were maintained as monolayers at 37°C and 5% CO_2, in high serum (HS) medium (DMEM-F12 supplemented with 15% FCS, 5µg/ml Insulin, and Penicillin/Streptomycin) with the medium being exchanged every other day. For differentiation cells were transferred into low serum (LS) medium, containing only 1% FCS.

Immunocytochemistry: Culltured cells were fixed for 10' with 4% paraformaldehyde. After treatment with Acetone/ Methanol (1:1), followed by washing with PBS, cells were blocked for 1h with goat serum (1:50) in PBS. Primary antibodies were applied over night at 4°C, followed by washing with PBS and biotin-coupled secondary antibodies (1:400 at RT). After washing with PBS, Peroxidase-coupled Streptavidin was used to label antibody tagged proteins by DAB-staining. After final washing and covering, imaging was performed using an Axiocam digital camera system mounted on an Axiophot microscope (Zeiss).

Western blot analysis: Cells were harvested in 5x Laemmli sample-buffer and protein concentration was densometrically determined. 5µg of total cellular protein was separated by polyacrylamide gel electrophoresis (8,5%). After tank-blot Western-transfer on PVDF-membranes, they were blocked with dry milk and incubated with primary antibodies (1:2000, 4°C) over night. After washing, rabbit specific peroxidase-coupled secondary antibodies (1:10.000) were applied for 90' at RT, and, after washing, ECL-detection was performed. For some experiments, blots were stripped with NaOH (0,1mol/l) for 15' and after blocking, were re-incubated with another primary antibody.

2D-Gel electrophoresis: Cells were harvested in 2x ampholyte sample buffer, and protein concentration was determined densitometrically. For the 1st dimension by isoelectric focusing, 30µg of total cellular protein was loaded on amphpholyte (pH3,5-10) containing polyacrylamide gel slices and separated electrophoretically. For the 2nd dimension, gel slices were equilibrated in Laemmli sample buffer and located on top of standard SDS polyacrylamide gels, where proteins were separated according their molecular weight. After tank-blot Western-transfer onto PVDF membrane, proteins were stained by immune incubation, and reactive protein spots were localized by ECL-detection and film autoradiography.

Digestion and LC-Q-TOF MS/MS analysis: Labeled protein spots were excised and digested as described previously*. Obtained peptides were reconstituted in an aqueous solution and introduced onto two consecutive nano-C18-reversed-phase chromatography columns using an auto CapLC sampler (Waters). The separated peptides were analyzed in a Q-TOF Ultima Global mass spectrometer (Waters) equipped with a nanoflow ESI Z-spray source in the positive ion mode. Data were acquired with the MassLynx (v4.0) software on a Windows NT PC and further processed using ProteinLynx Global Server (PLGS; v 2.2; Micromass). Obtained peak lists were searched online (http://www.matrixscience.com) by the MASCOT algorithm against the NCBI and SwissProt database. *Asif et al., 2010, Electrophoresis 31, 1947-1958.

Detection of mitochondrial activity by JC-1: Low density cultures of HIBCPP-cells on glass cover slips (Menzel) were maintained under low serum conditions and treated for 24h with 10µg/ml α-NG or α-NM, Na-azide of which has been removed by microdialysis using Amicon-Ultra filter units (Millipore). Cells were stained for 40 min with 2,5µg/ml JC-1, and after transfer into PBS red and green fluorescence was photographed using an Axiophot microscope equipped with epifluorescence (Zeiss) with FITC- and Rhodamine filter sets. Images were densitometrically evaluated with ImageJ, and red/green fluorescence ratio was calculated, indicating mitochondrial activity. In another series of experiments, cells were treated in parallel to α-NG also with the neuroleptic drugs Haloperidol (HAL; 0,1µmol/l), Risperidone (RIS; 1µmol/l), Clozapine (CLZ; 0,1µmol/l) (all from Sigma-Aldrich), and Olanzapine (OLA; 10µmol/l) (Lilly).

Crossreactivity of α-NG and α-NM in HIBCPP-cells: IHC and WB

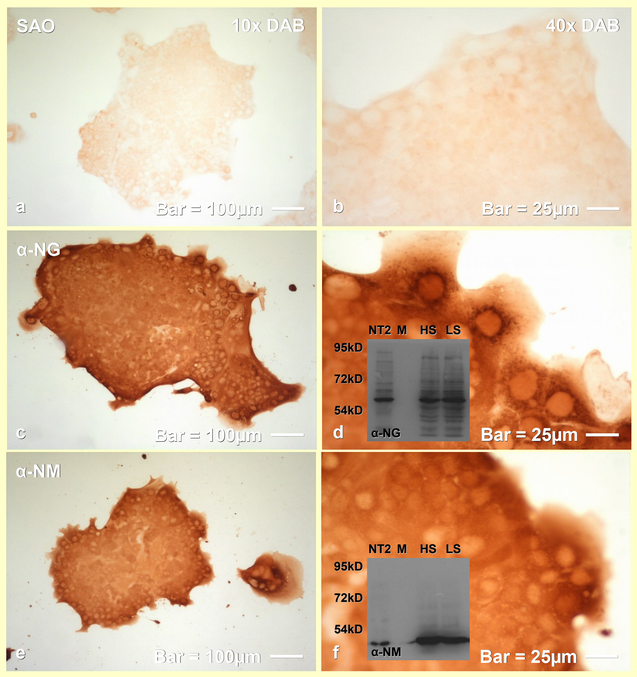
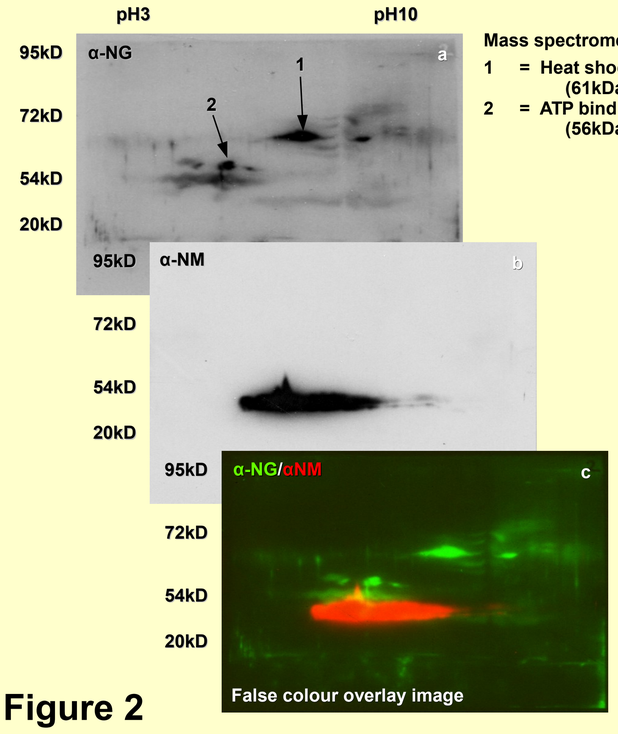


Figure 1

Crossreactivity of α-NG and α-NM in HIBCPP-cells: 2D-WB



Mass spectrometry:
1 = Heat shock protein 60 (61kDa, IEP = 5,7)
2 = ATP binding protein (56kDa, IEP =5,26)

Figure 2

Specific crossreactivity of α-NG and α-NM with HIBCPP, ATPB, and Hsp60: WB

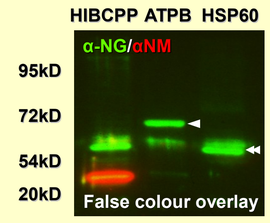
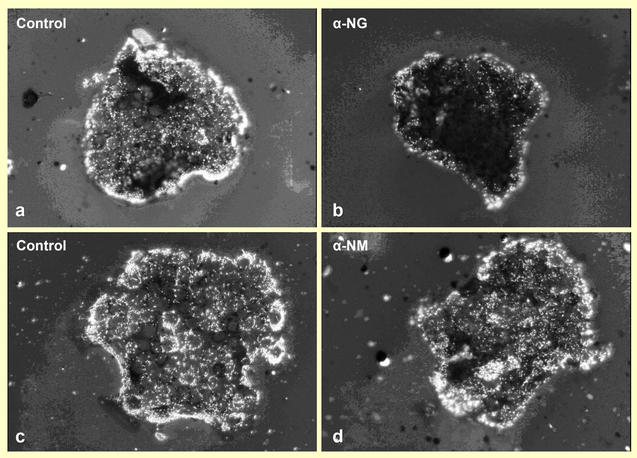


Figure 3

Effect of α-NG and α-NM on mitochondrial function in HIBCPP-cells: JC-1 staining



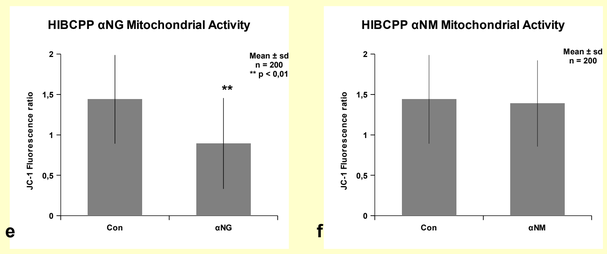
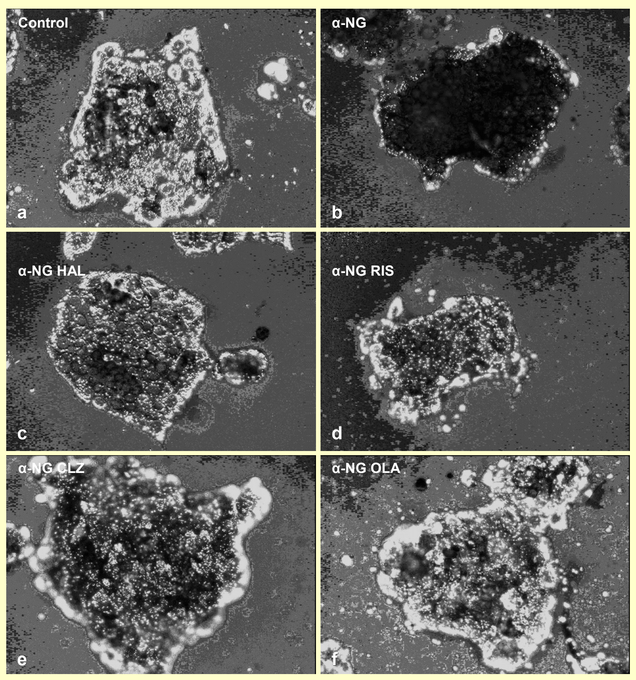


Figure 4

Reversal of the effect of α-NG on mitochondrial function in HIBCPP-cells by neuroleptic drugs: JC-1 staining



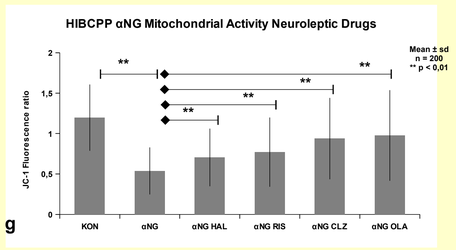


Figure 5

Results

Immunocytochemistry (Fig. 1c, d) demonstrated α-NG in HIBCPP cells, to label antigens located in an intracellular organelle. By Western blot analysis these antigens reappeared as a prominent band of around 60kDa, and several additional weaker bands of higher and lower molecular weights (Fig. 1d, inset). In contrast, antibodies directed to the closely related bacterium Neisseria meningitidis (α-NM) reacted with a cytoplasmic antigen (Fig. 1e, f), which in Western blot analysis appeared as a single band of a molecular weight of around 40kDa (Fig. 1f, inset). By 2D-immunoblot (Fig. 2), several α-NG-immunoreactive protein spots could be localized, and identified by LC-Q-TOF MS/MS analysis as human mitochondrial heat-shock protein Hsp60, and ATP binding protein ABP. For ABP, and Hsp60, this interaction could be confirmed by Western blot analysis using commercial protein samples (Fig. 3). Analysis of mitochondrial functioning by JC-1 staining revealed α-NG to result in a decreased red/green fluorescence ratio, whereas α-NM had no such effect (Fig. 4). Co-treatment with neuroleptic drugs revealed finally that the α-NG dependent reduction in mitochondrial activity (Fig. 5a, b) could be reverted significantly (Fig. 5g) by both typical and atpypical neuroleptic drugs like Haloperidol (HAL, Fig. 5c), Risperidone (RIS, Fig. 5d), Clozapine (CLZ, fig. 5e) and Olanzapine (OLA, Fig. 5f).

Conclusions

These results demonstrate that in human choroid plexus epithelium, antibodies directed to Neisseria gonorrhoeae interact with mitochondrial proteins like Hsp60, and ABP, functions of which suggest impaired ATP-production to be a consequence of this. This could directly affect choroid plexus transport functions, functional consequences of, i.e. with regard to schizophrenia pathogenesis will have to be studied at more detail in the future.

Acknowledgements

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