Impairment of neurite outgrowth in NGF-stimulated PC12 cells by antibodies directed to Neisseria gonorrhoeae, can be reversed by neuroleptic drugs *in vitro*

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Introduction

Neisseria gonorrhoeae (NG) is a Gram-negative bacterium, and a major cause for clinical and subclinical reproductive tract infections in women. In the case of pregnancy, prenatal maternal gonococcal infections are linked to an increased risk for the offspring to suffer from psychotic disorder in later life (Babulas et al., 2006, Am. J. Psychiatry 163, 927-929; Sørensen et al., 2009, Schizophrenia Bull. 35, 631-637). Since underlying mechanisms have not yet been clarified, we tested here the effects of a commercially available polyclonal antiserum from rabbit directed to Neisseria gonorrhoeae (αNG), on Nerve growth factor (NGF)-dependent neurite outgrowth in PC12-cells, a commonly used *in vitro* model for neuronal differentiation.

Materials&Methods

The rat pheochromocytoma cell line PC12 was maintained in Dulbeccos modified Eagle Medium (DMEM), supplemented with 10% fetal calf serum (FCS), 5% horse serum (HS), and Penicillin/Streptomycin (PS). Medium was exchanged twice per week. Prior to differentiation, cells were preincubated for one day with DMEM supplemented with 0,5% FCS, 0,25% HS and PS. For differentiation 10ng/ml NGF was added. Cells were treated with 10µg/ml of polyclonal antisera from rabbit directed to either Neisseria gonorhoeae (αNG), or Neisseria meningitidis (αNM). Na-azide was removed from the antisera using Amicon-Ultra microdialysis filter units (Millipore). Cells were also treated with antipsychotic drugs Haloperidol (HAL; 0,1µmol/l), Clozapine (CLZ; 0,1µmol/l), Olanzapine (OLA; 10µmol/l) and Risperidone (RIS; 1µmol/l). Cells were photographed with an Axiocam digital camera system and an Axiovert inverted microscope (both Zeiss). For every treatment and timepoint values were determined at 10 randomly chosen fields in 8 indepent experiments.

αNG specific antibodies impair neurite outgrowth in NGF-stimulated PC12 cells

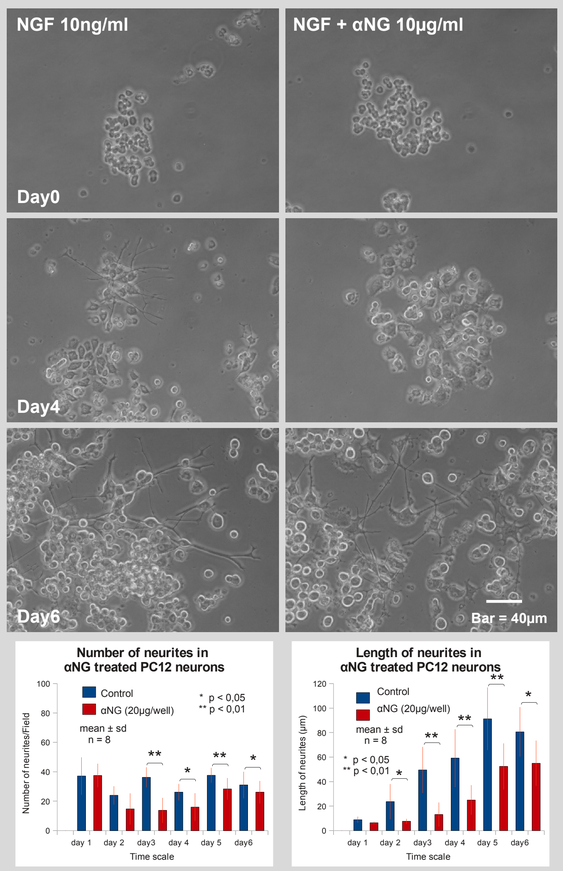

Figure 01

αNG but not αNM impair neurite outgrowth in NGF-stimulated PC12 cells

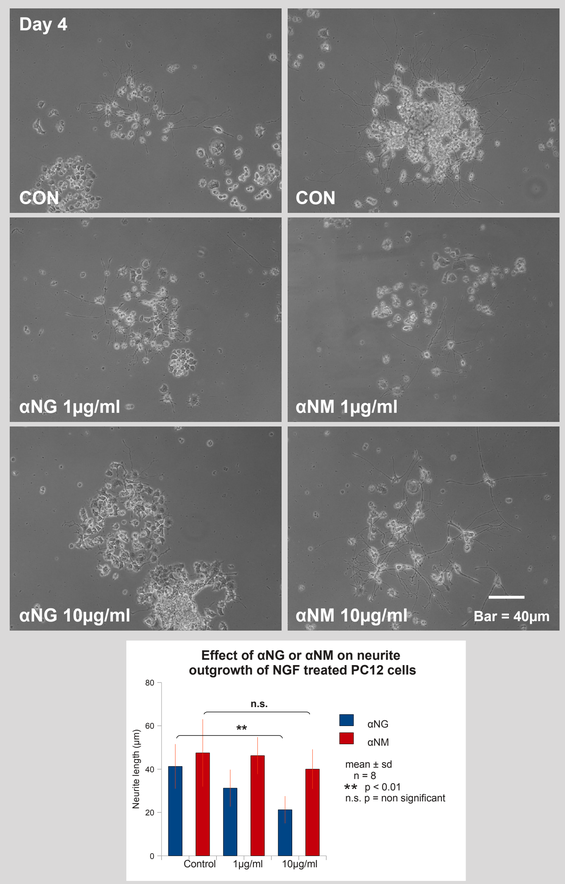

Figure 02

Neuroleptics revert the αNG dependent decrease in neurite length of PC12 cells

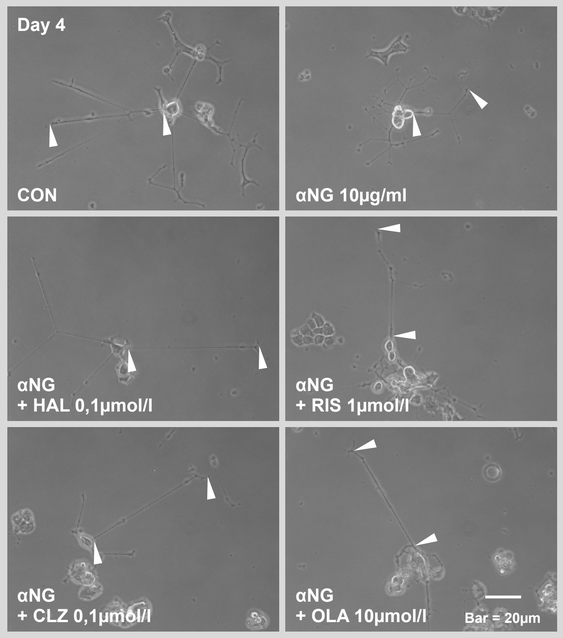

Figure 03

In terminally differentiated PC12 neurons αNG does not impair neurite integrity

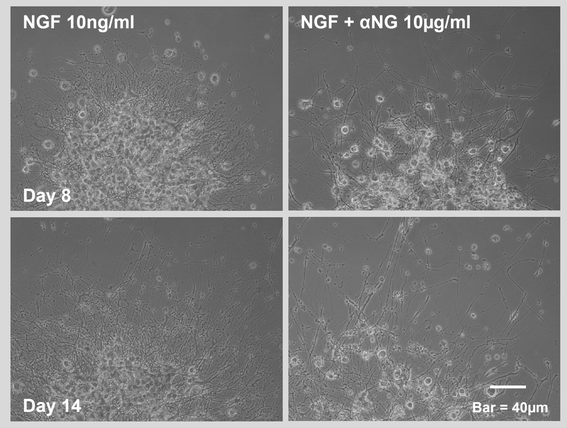

Figure 04

Results

Incubation of differentiating PC12 cells with 10µg/ml of αNG leads to a significant reduction of NGF dependent neurite outgrowth as compared to cultures treated with NGF alone (Fig. 01). In contrast, the same amount of αNM has no such effect (Fig. 02). Surprisingly, the reduction in neurite outgrowth caused in PC12 cells by αNG treatment, can be reversed by parallel application of the typical antipsychotic drug Haloperidol (HAL; 0,1 µmol/l or the atypical neuroleptic drugs Clozapine (CLZ; 0,1µmol/l) Olanzapine (OLA; 10µmol/l) and Risperidone (RIS; 1µmol/l) (Fig. 03). In contrast, neurites in terminally differentiated PC12-derived neuron like cells, are not affected by treatment with αNG (Fig. 04).

Conclusions

These results suggest that antibodies directed primarily to bacteria-specific antigens are able to crossreact with neuronal antigens, leading to impaired neurite outgrowth *in vitro*, and that this impairment can be overcome by parallel treatment of the cells with neuroleptic drugs. Although this mechanism might be relevant for a better understanding of the pathogenesis of psychotic disorders, its *in vivo* relevance, as well as the identity of the affected target molecule(s) remain to be clarified in the future.

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