Stoyan Borislavov Stoyanov Dr. sc. hum

## Role of methylglyoxal as mediator of hyperalgesia in early diabetic neuropathy

Geboren am12.02.1976 in Sofia, Bulgarien Diplom der Fachrichtung Biotechnologie am 09.2000 an der Universität Sofia, Bulgarien

Promotionsfach: Innere Medizin Doktormutter: PD Dr. Angelika Bierhaus

Glycation of proteins, nucleotides and phospholipids contributes to the development of late diabetic complications including the most debilitating diabetic neuropathy. Reactive intermediates of AGE formation such as glyoxal, methylglyoxal and other dicarbonyl compounds are detoxified by the glyoxalase-system. Survey of organs from wild type mice displayed particularly low levels of GLO-1 activity in sciatic nerves implying for high susceptibility of neuronal tissues to the damage of MG. Therefore we decided to study the role of MG-GLO-1-system as mediator of painful diabetic neuropathy.

Real Time PCR, immuno-staining and enzyme kinetics assays of tissues from sciatic nerves of wild type mice kept in diabetic state for 2 months illustrated down regulation of GLO-1 by at least 30 % compared to the controls. These data were confirmed in isolated DRGs from wild type mice cultured in medium with high glucose (30mM) displaying decreased transcription, expression and activity of GLO-1 under hyperglycaemic conditions.

Hot plate analgesia measurements demonstrated 25% increase in thermal nociception of wild type mice after 2 months of diabetes compared to healthy controls. Moreover determination of plasma concentration of MG revealed increase in the state of diabetes also paralleled by decrease in GLO-1 activity in these animals.

Inhibition of GLO-1 resulted in elevated thermal response latency in healthy wild type mice, which was also displayed by GLO-1-/+ mice. Consistently, overexpression of GLO-1 was able to restore thermal nociception to normal levels in diabetic animals and thus to implicate the significance of GLO-1 for prevention of development of diabetic neuropathy.

In addition treatment with 0.07mM MG alone was able to induce diabetes like hyperalgesic response in healthy wild type mice and thus implying for direct role of MG in the mechanism of pain. Immunoprecipitaion technique reveal binding of MG derived AGEs (MG-H1) antibodies to membrane fractions of sciatic nerves isolated from MG treated wild type mice and immunoprecipitated with Nav1.8-specific antibodies, indicating MG-mediated modification on this particular voltage-gated sodium channel.

Treatment of wild type mice with siRNA for Nav1.8 prevented MG induced thermal nociception. Moreover Nav1.8-/- mice were also protected from MG associated hyperalgesia, providing clear evidence of involvement of this channel in MG induced hyperalgeisa.

From our findings we can conclude that impairment of cellular defence system against methylglyoxal underlies the mechanism responsible for induction of hyperalgesia in early diabetic neuropathy.