Molecular Identification of Voltage-dependent Potassium Channels and Angiotensin II Receptors in the Diabetic Brain
Zeiter Stefan
Bachelor thesis, Bachelor of Sciences in Life Sciences, Molecular Bioanalysis

Principal: Prof Dr Pieles Uwe, FHWN (Muntenz)
Exposé: Dr Mutz Michael, Novartis (Basel)
Supervisor: Prof Dr Montiel-Herrera Marcelino, Universidad de Sonora (Hermosillo)

ABSTRACT
The frontal lobe (FL) is one of the four major lobes of the cerebral cortex in the mammalian brain and its dysfunction is responsible for several emotional disorders. This work is the first to show how hyperglycaemia changes the expression of angiotensin II (Ang II) receptors and potassium channels in the FL. Hyperglycaemia was induced in newborn Wistar rats (±12) by a single intraperitoneal injection of streptozotocin (n = 3). Control and hyperglycaemic FL slices were obtained, loaded with Fluor-AM and studied by means of confocal imaging. Basal intracellular Ca²⁺ concentration ([Ca²⁺]i) recordings revealed more random [Ca²⁺]i movement (17.8%) in the hyperglycaemic FL cells compared to healthy FL cells. Furthermore, bath application of 10 μM Ang II onto FL cells induced [Ca²⁺]i movement on 31% of control- and 35% of hyperglycaemic cells. Additionally, RT-PCR studies showed that, both, control and hyperglycaemic FL cells expressed only AT1 receptors and that hyperglycaemic FL cells expressed less transcripts for Ca²⁺-sensitive (BK) and delayed-rectifier (Kv4.2) K⁺ channels. These results may contribute to a better understanding of how Ang II receptors and K⁺ channels participate in the physiopathology of the diabetic brain.

INTRODUCTION
The FL is located at the front of each cerebral hemisphere and it is separated from the parietal lobe by a space between tissues called the central sulcus, and from the temporal lobe by a deep fold called the lateral sulcus [1]. The precentral gyrus, forming the posterior border of the FL, contains the primary motor cortex, which controls voluntary movements of specific body parts. The FL contains most of the dopamine sensitive neurons in the cerebral cortex and carries out higher mental processes such as thinking, decision making, and planning [2]. In this work, we aim to investigate the influence of hyperglycaemia on the physiology of the FL of Wistar rats because it has been suggested that hyperglycaemia affects the cognitive abilities of the human brain. Therefore, it could be expected to find molecular differences in the hyperglycaemic brain. This work contributes to the understanding of the changes in [Ca²⁺], expression of potassium channels and Ang II receptors in the postnatal hyperglycaemic FL.

RESULTS
First [Ca²⁺]i movements were measured in control and hyperglycaemic FLs. It was found that 54/143 control cells (n = 3 rats) showed regular basal activity in comparison with 62/249 hyperglycaemic cells (n = 3 rats). Also in the same cell population we determined random activities (defined as [Ca²⁺]i movements without an apparent pattern) in control (25/143 cells) and hyperglycaemic (84/249 cells) FL (Figure 1). Random activity in the hyperglycaemic brain was higher and with greater intensities (±16.2%). That could be considered as a clear indication that hyperglycaemia has altered its metabolic activity or the expression of Ca²⁺-permeable ion channels.

Ang II receptors change their expression during development. AT1 receptors are more abundant in adults and AT2 receptors are prevalent in embryonic and postnatal rats. In order to know if Ang II has an effect in [Ca²⁺], we used bath applied 10 μM Ang II in 143 control cells (n=3 rats) and 249 hyperglycaemic cells (n=2 rats). Both control (24/143 cells) and hyperglycaemic (31/249 cells) FLs responded with an increased [Ca²⁺], movements that recovered (Figure 2). Additionally Ang II decreased [Ca²⁺], movements in 16% of control cells and 11.6% of hyperglycaemic cells. In further analyses it was found that Ang II induced delayed responses after bath application in both control and hyperglycaemic FL in 17/143 and 43/249 cells, respectively and that when inward- and outward-Ca²⁺ movements responses are compared between them, all time Ang II outward Ca²⁺ movements were higher.

Comparison of basal recordings and Ang II induced responses in control and hyperglycaemic FL are showing different changes in [Ca²⁺]. With those two approaches it was found, that the diabetic brain behave differently of control brain.

CONCLUSION
In conclusion, this study shows that Ang II generates [Ca²⁺] movements by the activation of AT1 receptors in the control- and hyperglycaemic-FL brain. Additionally it is shown that hyperglycaemic FL cells express less K⁺ channels (BKα and Kv4.2) than the healthy FL cells. The results presented may provide a better understanding of how Ang II receptors and K⁺ channels participate in the physiology of the FL of the newborn rat brain. Further studies are required to investigate all interactions of diverse ion channels and chemical transmitters participating during development of the brain.

REFERENCES
[1] Selvaraj et al., 1998; Handedness identification from intertubercular sulcus of the humerus by discriminant function analysis
[3] Montiel & Garcia, 2010; Current profiles of astrocytes from the corpus callosum