DOCTOR OF PHILOSOPHY

Investigation of KATP channel function in response to metabolic and pharmacological manipulation, in the hypothalamic GT1-7 cell line

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Award date:
2014

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Candidates Declaration

I hereby declare that all results described in this thesis, unless otherwise stated, are entirely my own work. I further state that the composition of this thesis was performed by myself and none of the material has been submitted for any other degree. Lastly, I verify that all sources have been appropriately cited. This work was carried out in the Medical Research Institute, University of Dundee under the supervision of Professor Rory McCrimmon.

Elizabeth Haythorne

Supervisor's Declaration

I certify that Elizabeth Haythorne has completed 8 terms of experimental research and has fulfilled conditions of Ordinance 39, University of Dundee, such that she is eligible to submit the following thesis in application for the degree of Doctor of Philosophy.

Professor Rory McCrimmon

Summary

Animal and human studies have consistently demonstrated that recurrent hypoglycaemia (RH) blunts both hormonal and behavioral counter regulatory responses (CRR) to further episodes of hypoglycaemia. It is now well established that the brain is involved in regulating whole-body glucose homeostasis, including the CRR to hypoglycaemia. The aim of the current study was to investigate if adaptations occur, following RH, which are intrinsic to glucose-sensing neurons in the absence of synaptic/glial inputs or signals from the periphery. Utilising the GT1-7 hypothalamic mouse cell line as an *in vitro* model of homogenous glucose-excited neurons, the current study has demonstrated that recurrent low glucose exposure reprograms intracellular metabolism towards a "hypometabolic state". This result occurs in conjunction with an attenuated ability of the cells to hyperpolarise in response to low glucose and a reduction in the sensitivity of the K_{ATP} channel to activation by MgADP. In an attempt to reverse the changes observed in K_{ATP} channel activity, the SUR1-selective K_{ATP} channel opener, NN414, was applied chronically to GT1-7 cells. However, chronic K_{ATP} channel activation severely reduced channel conductance and sensitivity to activation by MgADP and further NN414 application. These results suggest that chronic activation of the K_{ATP} channel leads to the induction of a negative feedback mechanism to reduce channel activity. This may be in an attempt to maintain neuronal membrane potential within a physiological range. These results also suggest activation of central K_{ATP} channels during RH may be driving the resulting defective CRR. However, adaptations in metabolism following RH may also be altering the function of central K_{ATP} channels.

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Acknowledgements

Firstly I would like to thank my supervisors Professor Mike Ashford and Professor Rory McCrimmon for the opportunity of undertaking this research and providing supervision over the past 4 years.

I would like to thank all the members of the Ashford and McCrimmon labs, past and present, for providing an excellent research environment in which to carry out this project. I am especially grateful to Dr Lee Hamilton for words of encouragement, advice and for passing on his infectious enthusiasm for science. Thanks to my fellow PhD students, Susan, Geoff and Fiona for always being available to vent away the stresses of the PhD with a gin and a Nandos club. I would also like to thank my family for their support and encouragement over the past 4 years. They will be relieved that my 'eternal student' status is now over.

Finally I would like to thank John for his relentless support and faith in me. I am especially grateful for his patience and understanding when I was "just patching one more cell"!