Pharmacology of the ClC-1 Chloride Channel

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Discipline of Physiology

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Submitted for the degree of

Doctor of Philosophy

October 2009

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ABSTRACT

Clinical studies reported side effects of muscular spasms and muscle stiffness following the administration of clofibrate, a drug once used to treat hyperlipidaemia in patients. Experiments with clofibrate and its analogues in animal models showed it produced these myotonic symptoms in muscle by reducing the chloride conductance of the muscle membrane.

The effects of 2-(4-chlorophenoxy)propionic acid, an analogue of clofibric acid, was assessed on the rat ClC-1 channel (rClC-1). Racemic 2-(4-chlorophenoxy)propionic acid shifted the voltage dependence of rClC-1 activation to more depolarising potentials, a mechanism accounting for myotonic symptoms previously reported. Experiments with resolved enantiomers revealed that the effects recorded were due exclusively to S-(–) 2-(4-chlorophenoxy)propionic acid. The R-(+) enantiomer was ineffective at the concentrations tested. Further experiments with the compound at differing Cl^- concentrations in the extracellular solution suggested that S-(–) 2-(4-chlorophenoxy)propionic acid altered the gating of ClC-1 by decreasing the affinity of the binding site where Cl^- normally acts to 'gate' the channel.

Similarities in the effects reported for most dominant mutations in the *CLCN1* gene that lead to myotonia congenita and 2-(4-chlorophenoxy)propionic acid prompted experiments that introduced these point mutations in the human ClC-1 (hClC-1) gene to compare their mode of action to that of the drug. These mutations, F307S and A313T, predominantly altered the slow, or common, gate of the channel. Conversely, the effect of 2-(4-chlorophenoxy)propionic acid was predominantly on the fast gating process of hClC-1. A macroscopically similar effect therefore, can be produced by two different modes of action. Results suggested that both drug and mutations exert their action by

affecting the transition of the channel from its closed to open state subsequent to Cl^- binding.

Investigation of the interaction between rClC-1 gating and a further 25 compounds structurally related to clofibric acid identified a number of compounds effective at shifting the open probability of fast gating to depolarising potentials. Fewer were identified that influence slow gating. Some compounds affected both gating processes, however, none were identified which influenced slow gating alone. Ability to displace the voltage dependent activation of the fast gate appeared to depend largely on the lipophilicity of the molecules tested, indicating the importance of hydrophobic interactions between drug and channel protein.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Signed:

This Thesis is dedicated to the memory of

my friend and my brother

Massimo

5th April 1971 – 22nd Nov 1998

Though I don't think he ever fully understood what I did with my time, I know he was proud of me nonetheless...

ACKNOWLEDGMENTS

I would like to gratefully acknowledge the patient support and advice offered to me by my supervisors A. Prof. Michael Roberts, Prof. Allan Bretag, A. Prof Bernie Hughes and ultimately Dr. Grigori Rychkov. In particular I would like to express my sincere thanks to Michael Roberts and Grigori Rychkov whose friendship and guidance offered innumerable highs and instilled in me a passion for science and whose unwavering support enabled me to complete this undertaking, despite the many lows I also encountered.

I acknowledge the friendship and support of the many other members of the Cellular Physiology Laboratory with whom I had the opportunity to work over the years. In particular I would like to thank Brett Bennetts for his friendship, advice and his keen ability to help me keep things in perspective.

I would also like to acknowledge the contribution of Dr. Ted Lloyd from the Victorian College of Pharmacy for calculating the Log D values for the clofibric acid analogues presented in Chapter 4 of this thesis.

Finally, I would like to sincerely thank my family for supporting me and suffering with me during my studies, and also my friends whose encouragement at times made the process seem easier and more worthwhile.