ROLE OF GALANIN AND ITS ANTAGONISTS IN
EXPERIMENTAL ACUTE PANCREATITIS

A thesis submitted for the degree of Doctor of Philosophy

By

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7.4.5 Study groups
7.4.6 Parameters measured and statistical analyses
7.5 Results
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References
SUMMARY OF THESIS

The broad aim of the studies described in this thesis was to evaluate the role of neuropeptide galanin in acute pancreatitis (AP). Treatment of AP is mainly symptomatic and supportive and no definitive pharmacological therapy for this disease is currently available.

There are a number of studies in animal models of AP which demonstrate beneficial effect of a pharmacological agent in the management of AP. But most of these studies are limited to single species. The studies presented in the thesis evaluate the role of galanin and several of its antagonists in experimental AP in two different species. The initial part of the experimental work was performed in the possums, using a well established model of AP in the laboratory. Later, the experimental work has been carried out in the mouse.

The overall hypothesis was that galanin plays a major role in the onset and/or progression of AP.

In Chapter 2, the effect galanin or galantide administration, before and after AP induction on severity of AP in the possum model is described. The studies demonstrated that galantide decreased various indices of AP when administered prophylactically and therapeutically.
Chapter 3 outlines studies to determine if administration of galanin or galantide alters pancreatic vascular perfusion (PVP) during AP in the possum model. These studies suggested that in AP there is an initial fall in PVP, which is exacerbated by administration of galanin prior to onset of AP. Conversely, galantide administration prevented this decrease in PVP, and was associated with a rise in PVP throughout the duration of the experiment.

Chapter 4 describes preliminary studies on effect of galanin and galantide on pancreatic exocrine secretion. These demonstrated that galantide decreased hyperstimulated pancreatic exocrine secretion, but had no effect on the basal secretion.

The subsequent studies are carried out using the caerulein mouse model of AP. The hypothesis has been tested in three different strains of mice, including a galanin gene knock-out (KO) strain.

Chapter 5 outlines the effect galanin or galantide administration, before and after AP induction on the severity of AP in the caerulein mouse model. These studies revealed that galantide administration both prophylactically and therapeutically decreased the severity of AP in the mouse.

In Chapter 6, the galanin gene KO were used to further test the hypothesis. These studies revealed that AP was less severe in the galanin KO mice, thereby suggesting a role for endogenous galanin in the onset and/or progression of AP.
Chapter 7 describes the effects of various galanin antagonist on the severity of AP in the caerulein mouse model. These studies revealed that galantide and M35 have beneficial effects in AP, i.e. reduced the indices of AP, whereas C7 and M40 had complex effects.

Chapter 8 provides an overview of findings and discussion of their broader ramifications with future recommendations.

Overall, the studies have demonstrated that galanin plays a major role in AP and galanin antagonists may be of potential therapeutic value in the management of AP.
PUBLICATIONS FROM THE STUDIES IN THIS THESIS

Manuscripts

Brooke-Smith ME, Carati CJ, Bhandari M, Toouli J, Saccone GTP. Galanin in the regulation of pancreatic vascular perfusion. Accepted for publication in Pancreas, Jan 2008.

Due to intellectual property issues publications were delayed. Presently the following manuscripts are being prepared

Bhandari M, Thomas AC, Carati CJ, Toouli J, Saccone GTP. Galanin antagonism modifies hyperenzymemia and pancreatic vascular perfusion (PVP) changes induced by acute pancretitis (AP) in a possum model.


Bhandari M, Kawamoto M, Thomas AC, Carati CJ, Toouli J, Saccone GTP. The galanin knockout mouse is less susceptible to caerulein-induced acute pancreatitis.

Abstracts and Conference Presentations

Abstracts


Bhandari M, Thomas AC, Carati CJ, Kawamoto M, Brooke-Smith ME, Saccone GTP, Toouli J. Galanin antagonism reduces hyperenzymemia associated with acute pancreatitis (AP) in a possum model.


Bhandari M, Kawamoto M, Thomas AC, Carati CJ, Toouli J, Saccone GTP. The galanin knockout mouse is less susceptible to caerulein-induced acute pancreatitis. J HPB Surgery, in press.


Conference Presentations


Poster - Bhandari M, Thomas AC, Carati CJ, Kawamoto M, Brooke-Smith ME, Saccone GTP, Toouli J. (Presenter: M Bhandari). Galanin antagonism modifies acute pancreatitis (AP)-induced hyperenzymemia and pancreatic vascular


Poster - Bhandari M, Thomas AC, Carati CJ, Kawamoto K, Toouli J, Saccone GTP. Galanin antagonism modifies hyperenzymemia and pancreatic vascular perfusion (PVP) changes induced by acute pancreatitis (AP) in a possum model. (Presenter: GTP Saccone). Joint APA/IAP meeting, Chicago USA, November 2006. Poster of distinction


DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Mayank Bhandari, M.B.B.S., M.S
Date:
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I am indebted to my supervisors Professor James Toouli, Associate Professor Gino Saccone and Associate Professor Colin Carati for their continuous support, encouragement, constant assistance and critical and constructive feedback during my candidature. I wish to thank them for their useful discussion and comments regarding the preparation of this thesis and associated conference presentations. I am also grateful to Professor A Thomas for his help in histological analysis of slides.

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I would especially like to mention the helpful services of the staff in the Animal house and the staff in Medical Illustration and Media, thank you all for your help.

I wish to thank Dr. C.Ormandy from Garvan Institute of Medical Research, NSW for providing us with galanin gene knock- out mice.
I am also thankful to Flinders Technologies, Flinders university of South Australia, Bio Innovation SA and Flinders Medical Centre Research Foundation for the financial support provided for this project.

I wish to thank my wife, family and friends for their patience, encouragement and tireless support during the time taken to complete these studies and thesis.

Finally, I would like to dedicate this work to my beloved mother Mrs. Suman Bhandari.
ABBREVIATIONS

The following abbreviations are used throughout the text, figures and figure legends of this thesis.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>Acute pancreatitis</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>Ca++</td>
<td>Calcium</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CCK-8</td>
<td>Cholecystokinin octapeptide</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CVP</td>
<td>Central venous pressure</td>
</tr>
<tr>
<td>GAL-LI</td>
<td>Galanin-like immunoreactivity</td>
</tr>
<tr>
<td>GALR1</td>
<td>Galanin receptor 1</td>
</tr>
<tr>
<td>GALR2</td>
<td>Galanin receptor 2</td>
</tr>
<tr>
<td>GALR3</td>
<td>Galanin receptor 3</td>
</tr>
<tr>
<td>GMAP</td>
<td>Galanin message associated peptide</td>
</tr>
<tr>
<td>ICAM</td>
<td>Intercellular adhesion molecule</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>KO</td>
<td>Knock-out</td>
</tr>
<tr>
<td>LDF</td>
<td>Laser Doppler fluxmetry</td>
</tr>
<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NFK β</td>
<td>Nuclear factor kappa Beta</td>
</tr>
<tr>
<td>PD</td>
<td>Pancreatic duct</td>
</tr>
<tr>
<td>PDP</td>
<td>Pancreatic duct pressure</td>
</tr>
<tr>
<td>PVP</td>
<td>Pancreatic vascular perfusion</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>SEC</td>
<td>Secretin</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>VIP</td>
<td>Vasoactive intestinal polypeptide</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type</td>
</tr>
</tbody>
</table>
STRUCTURE OF THESIS

History of candidature

My candidature for this thesis commenced in April 2004 as a full time student. The literature review was surveyed during 2004. During this year preliminary studies were performed based on the findings of my predecessor, Mark Brook-Smith. Based on these preliminary studies the overall hypothesis and specific hypotheses were defined. The experimental studies were performed initially in the possum during the later half of 2004 to early 2006. Then the studies were undertaken in the mouse from 2006 to mid 2007 to further test the hypothesis. Subsequently during 2007-2008, the thesis was compiled for submission.

Thesis chapters

The structure of this thesis conforms to Flinders University guidelines. This thesis is presented in the following chapters.

Chapter 1 contains an overview of the relevant literature up to the time I completed experimental studies (mid 2007). The literature review has been updated to include key findings that aid in understanding of the pathophysiology of acute pancreatitis. Chapter 1 concludes with the presentation of general hypothesis, followed by the research aims.

Chapters 2-7 describe the experimental studies i.e. aims, methodology, analysis, statistical methods, results and discussion. Each chapter begins with a brief introduction.

Chapter 8 contains general discussion. The purpose of this chapter is to relate the findings to the original hypothesis. This section concludes with suggestions for future research.
Location of figures
To minimise disruption to the text, all figures are located near the end of each chapter.