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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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 through out 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 pancreatitis (AP) in a possum model.

Bhandari M, Thomas AC, Carati CJ, Kawamoto M, Toouli J, Saccone GTP. Galanin antagonism ameliorates hyperenzymemia and pancreatic necrosis in caerulein-induced acute pancreatitis in the mouse.

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

Saccone GTP, London JA, Woods CM, **Bhandari M**, Carati CJ, Brooke-Smith ME, Toouli J. Acute ethanol modulates pancreatic vascular perfusion in the Australian possum. Proceeding of the Falk Symposium No 143, poster # 42, 2004.

London JA, Woods CM, **Bhandari M**, Carati CJ, Brooke-Smith ME, Toouli J, Saccone GTP. Intragastric but not intravenous ethanol decreases pancreatic vascular perfusion in the Australian possum. Pancreas, 29 (4), 327, 2004.

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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.

Kawamoto M, **Bhandari M**, Thomas AC, Carati CJ, Toouli J, Saccone GTP. The galanin antagonist M35 but not M40 ameliorates caerulein-induced acute pancreatitis in a mouse model. J HPB Surgery, in press.

Bhandari M, Kawamoto M, Thomas AC, Carati CJ, Ormandy, CJ, Toouli J, Saccone GTP. Galanin knockout mice are less susceptible to caerulein-induced acute pancreatitis. Gastroenterology 132 (4), (Supplement 2), A-250, 2007.

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Kawamoto M, **Bhandari M**, Thomas AC, Carati CJ, Toouli J, Saccone GTP. The galanin antagonists galantide and M35 but not M40 ameliorate caerulein-induced acute pancreatitis in mice. Pancreatology, in press.

Conference Presentations

Poster - Saccone GTP, London JA, Woods CM, **Bhandari M**, Carati CJ, Brooke-Smith ME, Toouli J. Presenter: GTP Saccone). Acute ethanol modulates pancreatic vascular perfusion in the Australian possum. The Falk Symposium No 143 (Pancreatitis: Advances in pathobiology, diagnosis and treatment), Frieburg, Germany, October, 2004.

Oral - **Bhandari M**, Brooke-Smith ME, Carati CJ, Toouli J, Saccone GTP. (Presenter: M Bhandari). Galanin reduces pancreatic vascular perfusion (PVP) in the Australian possum. AHMR Congress, Sydney, NSW, November 2004.

Poster - London JA, Woods CM, **Bhandari M**, Carati CJ, Brooke-Smith ME, Toouli J, Saccone GTP. Presenter: GTP Saccone). Intragastric but not intravenous ethanol decreases pancreatic vascular perfusion in the Australian possum. APA Chicago, USA, November 2004.

Oral – **Bhandari M**, Thomas AC, Carati CJ, Toouli J, Saccone GTP. (Presenter: M Bhandari). Galanin antagonism ameliorates the severity of caerulein-induced acute pancreatitis in the mouse. Australasian pancreatic Club, Sydney, March, 2006.

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Oral - **Bhandari M**, Thomas AC, Carati CJ, Kawamoto M, Brooke-Smith ME, Toouli J, Saccone GTP. (Presenter: M Bhandari). Galanin antagonism reduces hyperenzymemia associated with acute pancreatitis (AP) in a possum model. ASMR (SA Branch) annual meeting June, 2006.

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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

perfusion changes in a possum model. Australian Gastroenterology Week, Adelaide, SA, October 11-14, 2006. Poster of Merit.

Poster - **Bhandari M**, Thomas AC, Carati CJ, Kawamoto K, Toouli J, Saccone GTP. Galanin antagonism ameliorates caerulein-induced acute pancreatitis (AP) in a mouse model. (Presenter: GTP Saccone). Joint APA/IAP meeting, Chicago USA, November 2006.

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Poster - Kawamoto M, **Bhandari M**, Thomas AC, Carati CJ, Toouli J, Saccone GTP. The galanin antagonist M35 but not M40 ameliorates caerulein-induced acute pancreatitis in a mouse model. (Presenter: J Toouli). A-PHPB, Fukuoka, Japan, March 2007.

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Poster - **Bhandari M**, Kawamoto M, Thomas AC, Carati CJ, Toouli J, Saccone GTP. Galanin receptor antagonist M35: a potential pharmacological treatment for acute pancreatitis. (Presenter: J Toouli). Digestive Diseases Week, Washington DC, USA, May, 2007.

Poster - Kawamoto M, **Bhandari M**, Thomas AC, Carati CJ, Toouli J, Saccone GTP. The galanin antagonists galantide and M35 but not M40 ameliorate caerulein-induced acute pancreatitis in mice. (Presenter: GTP Saccone). Digestive European Pancreatic Club, Newcastle-Gateshead, UK, July 2007.

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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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.

ANOVA	Analysis of variance
AP	Acute pancreatitis
BP	Blood pressure
BSA	Bovine serum albumin
Ca++	Calcium
CCK	Cholecystokinin
CCK-8	Cholecystokinin octapeptide
CNS	Central nervous system
CVP	Central venous pressure
GAL-LI	Galanin- like immunoreactivity
GALR1	Galanin receptor 1
GALR2	Galanin receptor 2
GALR3	Galanin receptor 3
GMAP	Galanin message associated peptide
ICAM	Intercellular adhesion molecule
IL	Interleukin
IV	Intravenous
KO	Knock-out
LDF	Laser Doppler fluxmetry
MPO	Myeloperoxidase
NO	Nitric oxide
NOS	Nitric oxide synthase
NFK ß	Nuclear factor kappa Beta
PD	Pancreatic duct
PDP	Pancreatic duct pressure
PVP	Pancreatic vascular perfusion
RNA	Ribonucleic acid

SEC	Secretin
SEM	Standard error of the mean
TNF	Tumour necrosis factor
VIP	Vasoactive intestinal polypeptide
WT	Wild type

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.