# EXOGENOUS PURINES INDUCE DIFFERENTIAL RESPONSES IN THE PROXIMAL AND DISTAL REGIONS OF THE SPHINCTER OF ODDI: PARTIAL CHARACTERISATION OF THE PURINERGIC RECEPTOR SUB-TYPES INVOLVED

A thesis submitted for the degree of Doctor of Philosophy

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# SUMMARY OF THESIS

The sphincter of Oddi (SO) is a neuromuscular structure located at the junction of the bile and pancreatic ducts with the duodenum. The primary functions of the SO are to regulate the delivery of bile and pancreatic juice into the duodenum, and to prevent reflux of duodenal contents into the biliary and pancreatic systems. Neural, hormonal or functional disturbances of biliary motility can lead to painful and sometimes life threatening clinical conditions, such as SO dysfunction and acute pancreatitis. Clearly understanding the regulation of biliary and duodenal motility patterns is necessary and may provide useful pharmacological sites for drug development to aid in the treatment of these diseases.

Spontaneous activity of the SO is regulated by complex interactions between the enteric nervous system, hormones, possibly interstitial cells of Cajal and other bioactive agents, together with modulation via neural reflexes between the duodenum, common bile duct/gallbladder, and stomach. Purines are one group of neurotransmitters/regulatory agents that have been shown to effect gastrointestinal motility, however their functions in the regulation of SO motility have not been elucidated.

The studies described in this thesis used *in vitro* organ bath techniques and *in vivo* preparations to determine the effects of exogenous purines on possum SO and duodenal motility. The possum SO has been extensively characterized and is an excellent model for motility studies. *In vitro*, exogenous adenosine was found to decrease spontaneous activity in both

the SO and duodenum. In contrast exogenous ATP induced both excitatory and inhibitory responses in the SO and duodenum. Interestingly, the adenosine and ATP-induced effects were predominantly exhibited by the proximal portion of the SO (proximal-SO), with no or little effect observed in the distal portion of the SO (distal-SO). These data support the hypothesis that the SO is comprised of different functional components that can act differently in response to certain stimuli, and highlights the importance of studying each of the SO components.

Agonists and antagonists, together with immunohistochemical studies, were used in an attempt to identify the P1 and P2 receptor sub-types responsible for mediating the adenosine- and ATP-induced responses. In the duodenum the adenosine-induced decrease in spontaneous activity was likely to be mediated by $A_{2A}$ and $A_3$ receptors, but the receptors mediating the proximal-SO response could not be identified. In the duodenum ATP induced a complex non-neural response consisting of a $P2X_1$, and $P2Y_2$ and/or $P2Y_4$ mediated immediate inhibition. This was followed by a return to baseline activity or small excitation. The response concluded with a late inhibitory response, likely to be mediated by $P2Y_1$ receptors, but the effects of other P2Y receptors could not be excluded. In contrast, ATP application to the proximal-SO evoked a partially neurally mediated early excitation, likely via P2X receptors, followed by an inhibition of activity, likely via activation of non-neural $P2Y_2$ and/or $P2Y_4$ receptors.

*In vivo* studies with exogenous application of adenosine and ATP to the SO activated neural pathways to produce increased motor activity.

Characterisation of these neural pathways found ATP and/or adenosine to activate excitatory cholinergic motor neurons. ATP also activated an inhibitory nicotinic/nitrergic pathway.

This is the first comprehensive investigation of the possible involvement of purines in the regulation of SO motility. These studies demonstrate that exogenous purines influence SO and duodenal motility, inducing complex neural and non-neural responses, acting via multiple P1 and P2 receptors. It now remains to be determined if endogenously released purines induce similar responses, together with elucidation and location of the receptor sub-types involved.

# PUBLICATIONS RESULTING FROM THE STUDIES IN THIS THESIS

## Manuscripts

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2003) $A_{2A}$ and $A_3$ receptors mediate adenosine-induced relaxation in spontaneously active possum duodenum *in vitro*. *British Journal of Pharmacology*, **138**:1333-1339.

**Woods, C.M.**, Mawe, G.M., Toouli, J. and Saccone, G.T.P. (2005) The sphincter of Oddi: Understanding its control and function. *Neurogastroenterology and Motility*, **17** (Suppl 1): 31-40.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2006) Exogenous purines induce differential responses in the proximal and distal regions of the possum sphincter of Oddi. *Autonomic and Autacoid Pharmacology* (in press).

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2006) Exogenous adenosine triphosphate and adenosine stimulate proximal sphincter of Oddi motility via neural mechanisms in the anesthetized Australiam possum. *Digestive Diseases and Sciences* (in press).

Note: these publications are included at the end of this thesis

## Abstracts and Conference Presentations

**Woods, C.M.** and Saccone, G.T.P. (2000) Exogenous adenosine has a biphasic effect on the spontaneous contractile activity of duodenal longitudinal muscle strips from the Australian brush-tailed possum. Purines 2000, Madrid, Spain, July 2000. *Drug Development Research*, **5**:79.

**Woods, C.M.** and Saccone, G.T.P. (2000) Exogenous adenosine inhibits spontaneous contractile activity in the Australian possum sphincter of Oddi *in vitro*. International Society of Autonomic Neuroscience, London, UK, July 2000. *Journal of Autonomic Nervous System*, **82**:82-83.

**Woods, C.M.** and Saccone, G.T.P. (2000) Exogenous adenosine triphosphate (ATP) has a complex effect on spontaneous contractile activity in the Australian possum sphincter of Oddi (SO) *in vitro*. International Society of Autonomic Neuroscience, London, UK, July 2000. *Journal of Autonomic Nervous System*, **82**:83.

**Woods, C.M.**, Toouli, T. and Saccone, G.T.P. (2000) Effects of adenosine and adenosine triphosphate (ATP) on sphincter of Oddi spontaneous contractile activity. The Surgical Research Society of Australasia, Adelaide, Australia, August 2000. *ANZ Journal of Surgery*, **71**:A115.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2000) Effect of exogenous adenosine on sphincter of Oddi and duodenal contractile activity *in vitro*. The Physiological and Pharmacological Society, Melbourne, Australia, November 2000. *Proceedings of the Australian Physiological and Pharmacological Society*, **31**:81P

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2001) Inhibitory effect of adenosine on spontaneous duodenal activity in the Australian Brush-tailed possum. XXXIV International Congress of Physiological Sciences, Christchurch, New Zealand, August 2001.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2002) Pharmacological characterisation of purinergic P1 receptors in duodenum longitudinal smooth muscle of the Australian possum. Digestive Diseases Week, San Francisco, USA, May 2002. *Gastroenterology*, **122**:A258.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2002) Characterisation of P1 receptors in the duodenum. 7$^{th}$ International Symposium on Adenosine and Adenine Nucleotides, Gold Coast, Queensland, Australia, September 2002.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2002) Exogenous adenosine triphosphate increases sphincter of Oddi activity *in vivo*. Australian Gastroenterology Week, Adelaide, Australia, October 2002.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2003) Sphincter of Oddi (SO) motility is increased by topical application of adenosine triphosphate (ATP). Australian Neuroscience, Adelaide, Australia, January 2003. *Proceedings of the Australian Neuroscience Society*, **14**: ORAL-02-07.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2003) Exogenous ATP increases sphincter of Oddi motility, acting via cholinergic and nitrergic pathways. Enteric Nervous System, Banff, Canada, July 2003. *Neurogastroenterology and Motility*, **15**: 216.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2004) Exogenous adenosine increases sphincter of Oddi motility, acting via cholinergic motor neurons. Digestive Diseases Week, New Orleans, USA, May 2004. *Gastroenterology* **126**: A-278.

**Woods, C.M.**, Toouli, J. and Saccone, G.T.P. (2004) Exogenous adenosine increases sphincter of Oddi motility, acting via cholinergic motor neurons. 6th World Congress, International Hepato-Pancreato-Biliary Association, Washington, USA, June 2004.

# DECLARATION

I certify that this thesis does not incorporate without acknowledgment 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.

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Charmaine M. Woods

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# ABBREVIATIONS

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

| | |
|---|---|
| ATP | Adenosine triphosphate |
| CBD | Common bile duct |
| CCK-8 | Cholecystokinin octapeptide |
| EFS | Electrical field stimulation |
| ENS | Enteric nervous system |
| EPSP | Excitatory post synaptic potential |
| GI | Gastrointestinal |
| IA | Intra-arterial |
| ICC | Interstitial cells of Cajal |
| IV | Intravenous |
| IPSP | Inhibitory post synaptic potential |
| L-NAME | $N^{\omega}$-nitro-L-arginine methyl ester |
| NANC | Non-adrenergic non-cholinergic |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| PBS | Phosphate buffered saline |
| SEM | Standard error of the mean |
| SO | Sphincter of Oddi |
| TTX | Tetrodotoxin |
| UTP | Uridine triphosphate |

**Note:** abbreviations for purinergic drugs are listed in **Table 1.2a** and **Table 1.2b**

# STRUCTURE OF THESIS

## History of candidature

My candidature for this thesis commenced in May 1999. The literature was surveyed during 1999 and preliminary studies were performed, leading to the development of the overall hypothesis and specific hypotheses. Experimental studies were performed from 2000-2002 on a full-time basis. Subsequently during 2003-submission the thesis was compiled on a part-time basis whilst undertaking full-time employment. During my candidature there has been considerable progress in understanding the role of purines in the small and large intestine, with regard to both secretory and motility functions, and in the localization of purinergic receptor sub-types. However there have been very few developments regarding the understanding of purines in the biliary tree.

## 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 commenced experimental studies (end 1999). This literature review has been updated to include key findings that aid in our understanding of biliary motility, but have bearing on the hypotheses generated or the experimental design. A major component of this chapter is a review of purinergic receptors, their agonists and antagonists. As information was limited regarding the use of these drugs in biliary or possum tissues, information

published prior to and during the period of experiments (pre1999-2002) is presented with regard to their use in the small intestine, specifically the guinea-pig ileum. Publications that directly relate to the interpretation of the data presented in this thesis that have been published since 2003 are included in the discussion section of the appropriate Results chapter.

Chapter 1 concludes with the presentation of the general hypothesis and specific hypotheses, followed by the research aims. It should be noted that technical limitations associated with the use of SO tissues resulted in the possum duodenum being used to evaluate drug concentration ranges. Therefore, the hypotheses and aims were expanded to incorporate a comparison between purinergic responses and receptors in the SO and duodenum.

Chapter 2 describes the methodology, experimental, analysis and statistical protocols used for the *in vitro* and *in vivo* studies.

Chapters 3-8 present the results of the experimental studies. Each chapter begins with a brief introduction, which builds on the information presented in the literature review and the findings presented in previous chapters. This is followed by the aims of the particular study, a brief methods section, and the results of the investigations. Each of these chapters contains an interpretation and comprehensive discussion of the data presented and refers to discussion in previous chapters to maintain continuity.

To aid interpretation of the data a number of summary diagrams are presented. Summary 1 summarises the *in vitro* investigations with adenosine, in both the SO and duodenum. Summary 2 summarises the *in vitro* investigations with ATP, in both the SO and duodenum. Summary 3 summarises the *in vivo* investigations of adenosine and ATP in the SO.

Chapter 9 contains a general discussion. As the previous chapters have included a comprehensive discussion of the data presented, the purpose of this final chapter is to relate the finings to the original hypotheses. This section concludes with suggestions for future research.

Appendix 1 contains the methodology and results of the immunohistochemical studies. These immunohistochemical studies were performed prior to the *in vitro* antagonist experiments in an attempt to identify the purine receptor sub-types present in the possum SO and duodenum, and their distribution. However due to non-specificity of the antibodies tested the results were equivocal and no conclusions could be drawn, but are presented for completeness.

The thesis concludes with a list of references to publications mentioned in the text.

## Location of figures

For minimize disruption to the text, all figures and tables are located in a group near the end or prior to the discussion of each chapter.