A Novel Gastrin Inhibitor In Sheep Abomasal Contents

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DAVID CRISPIN SIMCOCK

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ABSTRACT

Gastrin secretion was studied *in vitro* and *in vivo* in response to pharmacological agents and chemicals, as well as abomasal parasites and microbial products. The causes and effects of hypergastrinaemia, along with bacterial numbers and the presence of a gastrin secretion inhibitor in the abomasal contents of sheep infected with *Ostertagia circumcincta* were studied.

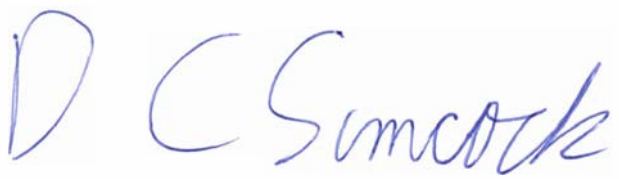
The pharmacology of the gastrin secretion from the unparasitised antrum was shown to be similar to that in monogastric animals. *In vitro* gastrin secretion by ovine antral segments was stimulated by Gastrin Releasing Peptide, carbachol and nicotine, but not adrenaline. Basal gastrin release was unaffected by somatostatin or Vasoactive Intestinal Polypeptide, but these reduced the gastrin response to stimulants. Gastrin secretion was also stimulated by amino acids, ammonia and acetate.

Hypergastrinaemia during *O. circumcincta* infection did not correlate well with decreased food intake or appear to affect parietal cell recovery. Serum gastrin concentrations correlated well with abomasal pH following adult *O. circumcincta* transplant, but poorly after larval infections. This suggests that other factors, such as inflammation and tissue damage, also affect gastrin secretion during abomasal parasitism. Anaerobic bacterial numbers in abomasal contents increased to near rumen levels when abomasal pH was 3.5 and above, but this did not affect serum gastrin concentrations. An inhibitor of *in vitro* gastrin secretion also started to appear in abomasal contents of pH3.5 and over, but did not have significant effects on *in vitro* gastrin secretion unless contents were pH4.5 and over. However, gastrin inhibitory activity in abomasal contents and serum gastrin levels were positively correlated, suggesting abomasal gastrin inhibitory activity has little effect on gastrin secretion *in vivo.*

Three competing factors were present in rumen fluid and rumen incubates: an inhibitor and a stimulant of secretion and an elimination factor. The stimulant was resistant to acid degradation, had a molecular weight below 3000 M_r and was hydrophilic. Both the elimination factor and the inhibitor were sensitive to acidity and hydrophobic and are likely to be proteinaceous.

STATEMENT

This is to certify that the work on which this thesis is based was carried out by the undersigned, and has not been accepted in whole or in part for any other degree or diploma. Assistance is specifically recorded in the Acknowledgements section bound with this thesis.



David Crispin SIMCOCK.
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LIST OF ABBREVIATIONS

α_2	Alpha adrenergic receptor subtype 2
Ach	Acetylcholine
ANOVA	Analysis of Variance
API	Adult Parasite Infected
$\beta_{2/3}$	Beta adrenergic receptor subtype 2 or 3
BRS-3	Bombesin receptor subtype 3
BSA	Bovine Serum Albumin
cAMP	cyclic Adenosine Monophosphate
°C	Degrees Celsius
CCK	Cholecystokinin
CCK_A	Cholecystokinin receptor class A
CCK_B	Cholecystokinin/gastrin receptor class B (gastrin receptor)
cells.mL^{-1}	viable cells per millilitre
CGRP	Calcitonin Gene Related Peptide
circ	circulation
cm	centimetre
cpm	counts per minute
CTR	control
D cell	somatostatin cell
DMPP	1,1-dimethyl-4-phenylpiperrazinium
EC	Enterochromaffin
ECL	Enterochromaffin like
EGF	Epidermal Growth Factor
e.p.g	eggs per gram
E/S	Excretory/Secretory
FEC	Faecal Egg Counts
g	grams
g	g force
g.L^{-1}	grams per litre
G17	Gastrin-17
G34	Gastrin-34
G17Gly	Glycine extended G17
G34Gly	Glycine extended G34
G-Gly	Glycine extended gastrins
G cell	Gastrin cell
GABA	Gamma Amino Butyric Acid
GIP	Gastric Intestinal Polypeptide
GLP	Glucagon Like Peptide
GRP	Gastrin Releasing Peptide
$GRPR_1$	GRP receptor subtype 1
$H_{2/3}$	Histamine receptor subtype 2 or 3
HBSS	Hank's Balanced Salt Solution
H. contortus	*Haemonchus contortus*
H. pylori	*Helicobacter pylori*
hGRP	human GRP
I cells	cholecystokinin containing cells
IL	Interleukin
INF	Interferon Gamma

iU	international unit
kDa	kiloDalton
kg	Kilogram
L	Litre
L3	Third stage larvae
LPI-1/2	Larval parasite infected, experiment 1 or 2
LTI	Larval Trickle Infected
M	Moles per litre
$M_{1/2/3}$	cholinergic receptor subtype 1, 2 or 3
mg	milligram
$mg.mL^{-1}$	milligrams per millilitre
$mg.kg^{-1}$	milligrams per kilogram
mL	millilitre
$mL.L^{-1}$	millilitres per litre
$mL.min^{-1}$	millilitres per minute
mM	millimolar
mOsm	milliosmoles
$mOsm.L^{-1}$	milliosmoles per litre
$\mu g.kg^{-1}$	micrograms per kilogram
µm	micrometres
µM	micromolar
µL	microlitre
µg	microgram
M_r	Molar ratio
mRNA	messenger RNA
NA	noradrenaline
NK	Neurokinin
NY	Neuropeptide Y
O. ostertagi/ circumcincta	*Ostertagia* spp.
p	probability statistic
PAM	Peptidylglycyl Amidating Mono-oxygenase enzyme
PBS	phosphate buffered saline
PC	Prohormone Convertase
pGRP	porcine GRP
PGE_2	Prostaglandin E_2
PHI	Peptide Histidine Isoleucine
pM	picomolar
PYY	Peptide YY
RIA	Radioimmunoassay
S. bovis	Streptococcus bovis
S cells	secretin containing cells
SD	standard deviation
SEM	standard error of the mean
SP	Substance P
SS	somatostatin
SST	somatostatin receptor
T. colubriformis	*Trichostrongylus colubriformis*
TGFα	Transforming Growth Factor Alpha

TNFα	Tumour Necrosis Factor Alpha
VIP	Vasoactive Intestinal Polypeptide
UNIANOVA	Univariate Analysis of Variance
v/v	volume to volume
w/v	weight to volume

PREFACE

Gastrin is a hormone secreted by the antrum of the stomach in monogastrics or the abomasum in ruminants. The classical action of gastrin is the control of acid secretion, for which it is the integration point for many stimulants and inhibitors. Gastrin has additional roles, notably the maintenance of gastric gland architecture and regulation of gastrointestinal motility. Hypergastrinaemia has been extensively studied in gastric diseases, particularly in humans with *Helicobacter pylori* infection, duodenal ulcers and pernicious anaemia. In duodenal ulcer patients, hypergastrinaemia is associated with the hypersecretion of acid. While most studies of gastrin secretion have been conducted in monogastric animals, the ruminant abomasum has similar architecture and functional cells to the stomach in other mammals (Murray *et al.*, 1970; Gurnsey *et al.*, 1985; Wathuta *et al.*, 1986) and its secretions also appear to be controlled by similar mechanisms (Lawton, 1995).

There is still debate regarding the causes and roles of hypergastrinaemia during abomasal nematode infection in ruminants. Fox *et al.* (1989a, b; 1993) have shown that in calves infected with *Ostertagia ostertagi*, hypergastrinaemia is very closely related to abomasal hypoacidity and a reduction in food intake. In fact, hypergastrinaemia is so closely correlated with the pathology of abomasal infection that it has been proposed as a diagnostic marker for parasitism. However, in sheep infected with *O. circumcincta*, hypergastrinaemia is not as closely related to abomasal hypoacidity. Notably, Lawton *et al.* (1996) observed that although hypergastrinaemia and abomasal hypoacidity develop in tandem, hypergastrinaemia persists when abomasal pH returns to normal levels. Thus, questions remain concerning the importance of other factors in stimulating gastrin secretion in the parasitised abomasum.

A particularly unusual observation in some parasitised sheep was a reversal of the hypergastrinaemia when abomasal pH exceeded pH5.5. This was suggested to be due to abomasal microbes inhibiting gastrin secretion. Microbial involvement in gastrin secretion during parasitism was supported by studies *in vitro*, in which a potent inhibitor of gastrin secretion was produced

when abomasal microbes were incubated aerobically (Haag, 1995; Lawton, 1995). This effect appears to be novel, as similar effects have not been reported in the numerous studies of *H. pylori* infections. The principal inhibitor of gastrin secretion in all species studied is somatostatin. Lawton (1995) suggested that the microbial inhibitor of gastrin secretion may be a somatostatin-like substance, however, the inhibitor reduced basal gastrin secretion in the *in vitro* antral preparation, unlike endogenous somatostatin released by pharmacological agents. Thus, it seems likely that the microbial factor which inhibited gastrin release appeared to act via a novel mechanism.

The primary objective was to determine the characteristics of the microbial inhibitor of gastrin secretion and whether it affected gastrin secretion during abomasal parasitism. To examine this, gastrin secretion was studied *in vitro* using tissue cultures and *in vivo* in sheep parasitised with *O. circumcincta*. *In vivo* studies were also used to examine the abomasal bacterial numbers and hypergastrinaemia during abomasal parasitism, as well as possible effects of hypergastrinaemia. *In vitro* experiments were also used to determine properties of the microbial inhibitory activity, and whether it was present in abomasal and rumen contents. *In vitro* and *in vivo* studies were combined to determine whether the microbial inhibitor of gastrin secretion affects gastrin levels during abomasal parasitism.