Acknowledgements

I would like to mention all those to whom I am so indebted for their help and support during my research work. I am deeply grateful to Prof. John Walker for not only accepting me as a Ph.D. student in the first place, but also for providing the solution to many seemingly intractable problems I encountered along the way. I am especially thankful to Dr C J Whelan (principle supervisor), without whose constant help and encouragement, none of my work would have been possible. Another great support and source of inspiration has been my co-supervisor, Prof. M E Parsons.

My study is a continuation of the work began by Deri Morgan, who helped me enormously in the early stages of my work. No research student can work for long without the ever-ready assistance from the technical staff and I have been extremely fortunate in this regard to be the recipient of so much help and encouragement from Brian Brandford and Diana Francis in particular amongst others in the technical staff. I have enjoyed working with my fellow Ph.D colleagues, Andrea Summers and Ken Ihenetu.

Lastly, I would like to thank my husband Mark Smith and his parents, Billy and Pam, for their constant support and help throughout, especially in the latter stages with the writing.

Abstract

Nicotine exerts a therapeutic effect in ulcerative colitis (UC) but the mechanism underlying this effect, is not clear. However, this effect may imply that nicotine has some, as yet to be discovered, effect on the immune system. The aim of the work described in this thesis was to characterise the nicotinic acetylcholine receptors (nAChRs) on human peripheral blood lymphocytes in term of receptor subtype. To achieve this, a combination of radioligand binding assays, pharmacological and molecular biological techniques were used.

The data obtained from the binding studies suggested that the presence of one binding site for (-)- nicotine on human peripheral blood lymphocytes with a K*d* 15 ± 5.759 nM (1.5 ± 5.759 x 10^{-8} M) and B*max* 2253 ± 409 sites/cell. The competition studies showed that ligands competing with [^{3}H]-(-)-nicotine were (-)-nicotine, epibatidine and α-bungarotoxin, while others ligands for nAChRs displaced radiolabelled nicotine in insignificant quantities. Thus, radioligand-binding experiments suggest that the binding site for nicotine on human peripheral blood lymphocytes is a nAChR containing α7 and possibly α4 or/and β2 containing nAChR subunits. No evidence was obtained to suggest the presence of a non-cholinergic nicotine receptor. Furthermore, considerable subject to subject variation in the specific binding of radiolabelled nicotine was observed. Because of this only tentative conclusions could be drawn from radioligand binding data.

Polymerase chain reaction (RT-PCR) was then used to demonstrate mRNA for the subunits of nAChRs suggested by radioligand binding studies. Data obtained show that the human peripheral blood lymphocytes tested, expressed mRNAs for α4, α5, α7, β2 neuronal nAChRs subunits and β1 muscle nAChR subunit.

Expression of the α5 mRNA subunit of nAChR was observed in the lymphocytes in each sample of lymphocytes tested. In contrast, the expression pattern of mRNAs for α4, α7, β1, and β2 mRNAs subunits of nAChRs, varied between individuals.

Finally, Western blot analysis was used to confirm that mRNA expression resulted in the expression of protein for nAChR subunits in human peripheral lymphocytes using monoclonal antibodies against α4, α5, α7, and β2 nAChR subunits, which had been detected by RT-PCR.

The results obtained from the Western blot analysis show that protein for α4, α5, and α7 nAChR subunits was expressed in most, but not all of the human peripheral blood lymphocyte samples tested and some of the bands obtained were faint. In contrast, protein for the β2 nAChR subunit was observed in a few samples tested and the bands were faint.

From the results obtained in this study, it is possible to conclude that human peripheral blood lymphocytes may contain nAChRs with subunit compositions of α4β2, α4β2α5, and/or α7. However, further studies are necessary to show whether or not the single binding site for nicotine demonstrated by radioligand binding experiments is due to one or all of these nAChRs.

Thus, the findings of the present study suggest the presence of nAChR on human peripheral blood lymphocytes. Nicotine and its effect may occur through these non-neuronal nAChRs mechanisms. Such a mechanism of action could account for the beneficial of nicotine in ulcerative colitis. Furthermore, a compound that acts on these receptors, but not on nAChRs found on other cells may have therapeutic utility in the treatment of inflammation.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

α-BTX	alpha bungarotoxin
$5HT_3$	5-hydroxytrytamine
ACh	acetylcholine
AChE	acetylcholine esterase
BE	bronchial epithelial cells
B*max*	the density of receptor site in particular tissue
bp	base pair(s)
BSA	bovine serum albumin
CD	Crohn's disease
cDNA	complementary DNA
ChAT	choline acetyltransferase
Ci	curie
CNS	central nervous system
Da	Dalton(s)
DEPC	diethyl pyrocarbonate
DHβE	dihydro-beta-erythroidine
DNA	deoxyribose nucleic acid
DNBS	dinitrobenzenesulphonic acid
DPM	disintegration per minute
DTT	dithiothretiol
EBI	European Bioinformatics Institute
ECL	enhance chemiluminescene
EDTA	ethylenediaminetetraacetic acid

FBS	foetal bovine serum
g	gram (s)
GABA	gamma-amino-butyric-acid
GI	gastro-intestinal
HBSS	Hanks' Balance Salt Solution
HEPES	N-2-Hydroxyethylpiperazine-N´-2-ethanesulfonic acid
HRP	horseradish peroxidase
IBD	inflammatory bowel disease
IC50	50% inhibitory concentration
IFNγ	interferon gamma
IgE	immunoglobulin E
IL-1	Interleukin-1
IL-2	interleukin 2
IP3	inositol-triphosphate
K*d*	dissociation equilibrium constant
kDa	kilodalton
L	litre (s)
M	molar (s)
mA	milliamp
mAChR	muscarinic acetylcholine receptor
mCi	millicurie
mg	milligram (s)
ml	millilitre (s)
mM	millimolar (s)
mRNA	messenger ribonucleic acid

mV	milivolt (s)
nAChR	nicotinic acetylcholine receptor
NBTS	national blood transfusion services
NCBI	National Centre for Biotechnology Information
nM	nanomolar
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PMN	polymorphonuclear
PVDF	electro blotting membrane
RNA	ribose nucleic acid
rpm	revolutions per minute
RT-PCR	reverse transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
TAE	Tris-acetic acid-EDTA buffer
TEMED	N,N,N´,N´-tetramethylenediamine
TNBS	trinitrobenzenesulphonic acid
TNFα	tumour necrosis factor alpha
UC	ulcerative colitis
µg	microgram (s)
µl	microlitre (s)
µM	micromolar (s)
UV	ultra violet
V	volts

LIST OF CHEMICALS AND REAGENTS

Chemical/Reagent	Supplier
Access RT-PCR system	Promega
α-BTX	Sigma
Acetic acid	Sigma
Acetone	Fisher
Acrylamide	BDH
Agarose	National Diagnostics
Aprotinin	Sigma
Atropine	Sigma
Ammonium persulphate	Sigma
AMV reverse transcriptase	Promega
Anti- rat IgG HRP conjugate	Merck Biosciences
Anti- mose IgG HRP conjugate	Merck Biosciences
Bis-acrylamide	BDH
β-Mercaptoethanol	Sigma
Bromophenol blue	Sigma
Buffy coat	National Blood Transfusion Services
Carbachol	Sigma
Chloroform	Sigma
Coomassie brilliant blue	Sigma
Cytisine	Sigma
D-glucose	Sigma

Diethyl pyrocarbonate	Sigma
Dithiothreitol	BDH
ECL kit	Amersham Pharmacia Biotech
EDTA (sodium salt)	BDH
Epibatidine	Torcris
Ethidium bromide	Sigma
Ficoll 400	Sigma
Foetal bovine serum	Life Technologies
General sterile plastics	Greiner Labortecknik
Glycerol	Sigma
[^{3}H]-(-)-nicotine	NEN Life Sciences
HEPES	BDH
Hexamethonium	Sigma
Histopaque®-1077	Sigma
Hyperfilm	Ammersham Pharmacia Biotech
IMS	Fisher
Isopropanol	Fisher
KH_2PO_4	BDH
Leupeptin	Sigma
L-glutamine	Life Technologies
Liquiscint scintillation fluid	National Diagnostics
MAB for α4, α5, β2 nAChR subunits	Covance Research products
MAB for α7 nAChR subunit	Research Diagnostics
NaCl	BDH
Na_2HPO_4	BDH

(-)-nicotine	Sigma
Nitrocellulose membrane	BDH
Nuclease free water	Promega
PBS tablets	Sigma
Penicillin	Life Technologies
Powdered milk (non-fat dried milk)	Marvell
Perfect protein™ HRP	Merck Biosciences
Prestained protein marker	Bio-rad
Primers	Invitrogen
RNA-STAT 60™	Biogenesis
RPMI-1640	Sigma
SDS	Fisher
Skatron 12 well micro tubes	Camo Ltd
Streptomycin	Life Technologies
Sucrose	BDH
TEMED	Sigma
Tissue culture flasks ($75ml^3$)	Becton Dickinson
Tris base	BDH
Trypan Blue	Sigma
Tween-20	Sigma
Whatman GF/B filters	Sigma