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ADENINE NUCLEOTIDE METABOLISM

IN

HUMAN BLOOD PLATELETS

A Thesis Presented in Partial Fulfilment
of the Requirements for the Degree of

MASTER OF SCIENCE in CLINICAL BIOCHEMISTRY

at

MASSEY UNIVERSITY
NEW ZEALAND

BRUCE MATHEW FARNDAL

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At present, a skeletal muscle biopsy provides the most specific test for susceptibility to Malignant Hyperthermia (MH). This procedure is unsuitable for large scale screening of individuals and a simpler, less invasive test to distinguish MH-susceptible (MHS) people from those not possessing the genetic defect, is highly desirable.

Platelets contain a calcium-activated contractile system, a calcium-storing and releasing system, and an active ATP-generating system. It is thus logical to assume that the same processes occur in platelets as those in muscle during MH-induced accelerated metabolism. In this research, [8-¹⁴C]adenine incorporation into blood platelet adenine nucleotides was investigated with a view to using differences between platelets from normal and MHS individuals as the basis for a clinical test.

It was assumed that under resting and/or halothane-stimulated conditions, nucleotide turnover in MHS platelets is significantly abnormal, and that the turnover abnormality is reflected in differences in adenine incorporation to platelet nucleotides via the salvage pathway.

MHS platelets took up less adenine and assimilated it into nucleotides at a slower rate than normal platelets. However, after two hours, 20% more labelled ATP was extracted from MHS platelets than normal, with a concomitant decrease in ADP levels. Halothane had little effect on normal platelets but caused a 10% decrease in incorporation into ATP in MHS platelets. AMP labelling was lower than normal in MHS platelets, indicating increased deamination of this

nucleotide.

Specific radioactivities of nucleotides were not measured since [¹⁴C]adenine distributes evenly among metabolic ATP, ADP, and AMP; therefore, the total radioactivities were used as a measure for the levels of adenine nucleotides within the metabolic pool.

From the limited number of individuals screened, results suggest that MHS platelets have a higher basal ATP turnover rate than normal. When challenged with halothane the adenylate energy charge decreased, causing an increased nucleotide turnover rate which in turn led to a decreased ATP level due to the increased deamination of AMP. The appearance of more hypoxanthine and inosine than normal in the extraplatelet medium is consistent with the above sequence of events.

The platelet-halothane bioassay displays a limited ability to distinguish between normal and MHS individuals and may have the potential to become a less invasive equivalent to the "ATP-depletion test" in muscle.

I would like to thank my supervisor, Dr Bob Greenway, for sharing his time and wisdom with me for the duration of this research, and for tolerating the unavoidable encroachment of extra-university activities on my studies.

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	PAGE
ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS.....	iv
LIST OF FIGURES.....	viii
LIST OF TABLES.....	x
LIST OF ABBREVIATIONS.....	xi

CHAPTER 1: INTRODUCTION.

1.1 MALIGNANT HYPERTHERMIA.....	1
1.1.1 General Features.....	1
1.1.2 Halothane and its Action.....	9
1.1.3 Biochemical Defect of Malignant Hyperthermia.....	11
1.1.4 Biochemical Events of Malignant Hyperthermia.....	13
1.2 BLOOD PLATELETS.....	16
1.3 PLATELET ADENINE NUCLEOTIDES.....	22
1.3.1 Introduction.....	22
1.3.2 Adenine Nucleotide Formation in Blood platelets.....	24
1.3.3 Adenine Nucleotide Compartments in Blood Platelets.....	30
1.3.4 Metabolism of Platelet Cytoplasmic Nucleotides.....	32
1.3.5 Protein-Bound Nucleotides in Blood Platelets.....	34
1.3.6 Adenylate Energy Charge.....	35
1.4 PREANAESTHETIC DIAGNOSIS OF MALIGNANT HYPERTHERMIA.....	37
1.4.1 Introduction.....	37
1.4.2 Non-Invasive Studies.....	37
1.4.3 Invasive Studies.....	40
1.4.4 Platelets in Malignant Hyperthermia.....	42
1.5 THE PRESENT RESEARCH.....	45

CHAPTER 2: MATERIALS AND METHODS.

2.1 CHEMICALS.....	46
2.1.1 Radioactive Chemicals.....	46
2.1.2 Halothane.....	46
2.1.3 Solvents.....	46
2.1.4 Other Chemicals.....	46
2.2 SUBJECTS FOR STUDY.....	47
2.2.1 Normal Subjects.....	47
2.2.2 Malignant Hyperthermia-Susceptible Subjects.....	47
2.3 CHROMATOGRAPHY.....	48
2.4 COLLECTION OF BLOOD.....	49
2.4.1 Method 1.....	51
2.4.2 Method 2.....	52
2.5 PREPARATION OF PLATELETS.....	53
2.5.1 Introduction.....	53
2.5.2 Platelet-Rich Plasma.....	55
2.5.2.1 Method 1.....	56
2.5.2.2 Method 2.....	57
2.6 PLATELET COUNTING.....	59
2.6.1 Introduction.....	59
2.6.2 Procedure.....	60
2.6.3 Results and Discussion.....	61
2.7 PREPARATION OF PLATELET EXTRACTS.....	62
2.7.1 Introduction.....	62
2.7.2 Procedure.....	63
2.7.2.1 Holmsen and Rozenberg (1968a).....	63
2.7.2.2 Solomons <u>et al.</u> (1978).....	64

2.7.2.3 Rao <u>et al.</u> (1981).....	65
2.8 pH ADJUSTMENT OF PLATELET EXTRACTS.....	66
2.8.1 Introduction.....	66
2.8.2 Procedure.....	67
2.8.3 Discussion.....	68
2.9 DETECTION OF NUCLEOTIDES.....	69
2.9.1 Introduction.....	69
2.9.2 Paper Chromatography.....	72
2.10 RADIOCHROMATOGRAM SCANNING.....	74
2.11 SCINTILLATION COUNTING.....	78

CHAPTER 3: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.

3.1 INTRODUCTION.....	81
3.2 CHROMATOGRAPHIC CONDITIONS.....	82
3.2.1 Rao <u>et al.</u> (1981).....	82
3.2.1.1 System A.....	82
3.2.1.2 System B.....	83
3.2.2 Solomons <u>et al.</u> (1984b).....	83
3.3 RESULTS.....	84
3.3.1 Evaluation of Precolumn Treatment.....	84
3.3.2 Nucleotide Standards.....	85
3.3.3 Platelet Extracts.....	88
3.3.3.1 Rao <u>et al.</u> (1981).....	88
3.3.3.2 Solomons <u>et al.</u> (1984b).....	92
3.4 STABILITY OF NUCLEOTIDES DURING STORAGE.....	93

CHAPTER 4: RESULTS.

4.1 ANALYSIS OF STOCK ADENINE.....	97
4.2 EVALUATION OF THE METHOD OF HOLMSEN AND ROZENBERG (1968a)....	97
4.3 EVALUATION OF THE METHOD OF SOLOMONS <u>et al.</u> (1978).....	102
4.4 ASSESSMENT OF PLATELET INTEGRITY.....	105
4.5 REANALYSIS OF STOCK ADENINE.....	106
4.6 EVALUATION OF THE METHOD OF RAO <u>et al.</u> (1981).....	108
4.7 EFFECT OF ADENINE.....	111
4.8 RECOVERY OF RADIOACTIVITY.....	115
4.9 EFFECT OF HALOTHANE.....	117
4.9.1 Dose-Response.....	117
4.9.2 Effect on Normal Platelets.....	121
4.10 TIME-COURSE EXPERIMENTS.....	124
4.10.1 Normal Platelets.....	124
4.10.2 Malignant Hyperthermia-Susceptible Platelets.....	126
4.10.3 Recovery of Radioactivity.....	143
4.10.4 Acid-insoluble Material.....	146
4.10.5 Supernatant Plasma.....	146

CHAPTER 5: DISCUSSION..... 153

REFERENCES..... 170

FIGURE	PAGE
1.1 Cross-sections through a blood platelet.....	17
1.2 Role of platelets in haemostasis.....	21
1.3 Synthesis of purine nucleotides in platelets.....	26
1.4 Synthesis of AMP in platelets.....	28
1.5 Adenine nucleotide metabolism in platelets.....	29
2.1 Preparation of platelet-rich plasma.....	58
2.2 Titration of platelet extracts using a blood-gas analyser.....	70
2.3 Titration of platelet extracts using pH indicator paper.....	71
2.4 Example of a paper chromatogram.....	73
2.5 Example of a radiochromatogram scan.....	76
2.6 Radioactivity of chromatogram determined by liquid-scintillation counting.....	77
3.1 Elution of platelet extract from Sep-Pak.....	86
3.2 Separation of nucleotide standards using system A and the method of Rao <u>et al.</u> (1981).....	87
3.3 Separation of nucleotide standards using system B and the method of Rao <u>et al.</u> (1981).....	89
3.4 Separation of platelet extract components using system B and the method of Rao <u>et al.</u> (1981).....	90
3.5 Separation of nucleotide standards using system B and the method of Solomons and Masson (1984b).....	94
3.6 Separation of platelet extract components using system B and the method of Solomons and Masson (1984b).....	95
3.7 Stability of ATP stored for six weeks.....	96

4.1	Components of platelet extracts prepared with the method of Holmsen and Rozenberg (1968a).....	100
4.2	Effect of adenine on nucleotide profiles.....	114
4.3	Recovery of radioactivity during preparation of platelet extract.....	116
4.4	Effect of halothane on nucleotide profiles.....	119
4.5	ATP in normal and MHS platelets.....	129
4.6	ADP in normal and MHS platelets.....	130
4.7	AMP in normal and MHS platelets.....	131
4.8	Hypoxanthine in normal and MHS platelets.....	132
4.9	Adenine in normal and MHS platelets.....	133
4.10	Ratio of ATP/ADP in normal and MHS platelets.....	135
4.11	Adenylate energy charge in normal and MHS platelets.....	137
4.12	ATP in normal and MHS platelets(b).....	140
4.13	ADP in normal and MHS platelets(b).....	141
4.14	AMP in normal and MHS platelets(b).....	142
4.15	Hypoxanthine in normal and MHS platelets(b).....	143
4.16	Recovery of radioactivity from acid-soluble extracts of in normal and MHS platelets.....	147
4.17	Acid-insoluble material in extracts of normal and MHS platelets.....	150
4.18	Nucleotide profile of supernatant plasma from normal platelets.....	151
4.19	Nucleotide profile of supernatant plasma MHS platelets.....	152

TABLE

1.1 Documented cases of MH in New Zealand.....	4
1.2 MH-triggering agents.....	8
1.3 Proportions of total platelet acid-soluble mono-, di- and triphosphates.....	23
2.1 Blood collection and processing by major research groups.....	50
4.1 Radioactive components of platelet extracts prepared by the method of Holmsen and Rozenberg (1968a).....	99
4.2 Radioactive components of platelet extracts prepared by the method of Solomons <u>et al.</u> (1978).....	103
4.3 Radioactive components of platelet extracts prepared by the method of Rao <u>et al.</u> (1981).....	110
4.4 Effect of adenine on nucleotide profiles.....	113
4.5 Effect of halothane on nucleotide profiles.....	118
4.6 Effect of halothane on normal platelets.....	122
4.7 Time-course experiments with normal platelets in the absence of halothane.....	127
4.8 Time-course experiments with normal platelets in the presence of halothane.....	128
4.9 Ratio of ATP/ADP in normal and MHS platelets.....	134
4.10 Adenylate energy charge in normal and MHS platelets.....	136
4.11 Time-course experiments with MHS platelets in the absence of halothane.....	138
4.12 Time-course experiments with MHS platelets in the presence of halothane.....	139

4.13 Recovery of radioactivity from extracts of normal and MHS platelets.....	146
4.14 Radioactivity in acid-insoluble material from normal and MHS platelets.....	149

AEC	- Adenylate Energy Charge
ADP	- Adenosine Diphosphate
AMP	- Adenosine Monophosphate
ATP	- Adenosine Triphosphate
BTB	- Bromthymol Blue
CCD	- Citrate-Citric Acid-Dextrose
CPD	- Citrate-Phosphate-Dextrose
CPK	- Creatine Phosphokinase
CPM	- Counts Per Minute
HPLC	- High-Performance Liquid Chromatography
HX	- Hypoxanthine
MH	- Malignant Hyperthermia
MHS	- Malignant Hyperthermia-Susceptible
PCA	- Perchloric Acid
PPO	- 2,5-diphenyloxazole
POPOP	- 1,4-bis-2-(5-phenyloxazolyl)benzene
mol	- mole(s)
mmol	- millimole(s)
M	- Molar
L	- Litre
ml	- millilitre
cm	- centimetre
min	- minute
μ l	- microlitre(s)
mCi	- millicuries
$^{\circ}$ C	- degrees Celcius