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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 FARNDALE

1985

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-^{14}C]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 concomittant 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 [^{14}C]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.

In the provision of patients susceptible to Malignant Hyperthermia (MH), Ian Anderson (formerly Senior Lecturer in the Faculty of Veterinary Science at Massey University), and John Stokes (Anaesthetist at Palmerston North Public Hospital) gave vital assistance. The enthusiasm of Drs Anderson and Stokes for research into the diagnosis of MH-susceptibility was evident from the helpful and informative discussions we shared.

Equally enthusiastic were the patients themselves, and their interest in MH research and cooperation in providing blood samples was very much appreciated. Thanks are also due to the people who did not turn away as I approached clutching a handful of vacutainer tubes and a twenty-one gauge needle. These people kindly donated blood samples from which a range of normal values was obtained.

Finally, I would like to express my gratitude to the staff and students of the Department of Chemistry and Biochemistry at Massey University for their friendship and constant encouragement and assistance, and to my Equine friends for always being there.

PAGE

CHAPTER 2: MATERIALS AND METHODS.

FIGURE PAGE

TABLE

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
μl	- microlitre(s)
mCi	- millicuries
^{o}C	- degrees Celcius