ACETALDEHYDE METABOLISM IN MAMMALS

A thesis presented in partial
fulfilment of the requirements
for the degree of

Doctor of Philosophy
in Biochemistry
at
Massey University
New Zealand

Allan Robert STOWELL
1977

ABSTRACT

To determine the pharmacological importance of acetaldehyde in the actions of ethanol, this study was planned to define the levels of free acetaldehyde occurring in human blood during the oxidation of ethanol in the body.

Peripheral venous blood acetaldehyde levels were determined by direct assay while pulmonary blood levels were estimated from breath acetaldehyde levels by employing the blood:air partition ratio for acetaldehyde at 37°C of 189 ± 18. Pulmonary blood acetaldehyde levels were determined to obtain values for (a) acetaldehyde output from the liver and (b) acetaldehyde levels in blood likely to be reaching the brain.

Sensitive enzymic methods for the determination of acetaldehyde in human blood and breath samples were developed, allowing levels of acetaldehyde as low as 0.5 μmoles/l and approximately 0.2 nmoles/100ml to be measured in blood and breath samples respectively, using either yeast or sheep-liver aldehyde dehydrogenases. The methods were developed to be operated in semi- or fully automated modes and involved continuous-flow distillation of samples with fluorometry.

Two methodological problems associated with the direct assay of acetaldehyde in blood were studied. These were (a) the production of acetaldehyde during the deproteinization of ethanol-containing blood with perchloric acid and (b) the rapid disappearance of acetaldehyde in blood samples.

It was found that over 90% of the acetaldehyde produced during the processing of ethanol-containing human blood for assay originates from reactions occurring when red blood cells, as distinct from plasma, are treated with perchloric acid.

The disappearance of acetaldehyde which had been added to human blood samples was found to result from the rapid metabolism of acetaldehyde to acetate by red cells. By contrast, acetaldehyde formed from *in vivo* ethanol metabolism did not appear to be metabolized significantly in blood samples. It was suggested that acetaldehyde formed *in vivo* may be bound to blood components.

While human subjects were metabolizing standard 1 g/kg doses of ethanol, breath acetaldehyde concentrations were found to range from 0.5 to 10.0

nmoles/100ml while peripheral whole blood acetaldehyde levels ranged from 0 - 12 μM and peripheral plasma levels ranged from 0 - 3 μM. Estimated pulmonary blood and hepatic venous blood levels of acetaldehyde fell within the ranges 0.9 - 19 μM and 4.5 - 95 μM respectively. The changes in the acetaldehyde concentrations of blood and breath during the metabolism of ethanol did not follow any identifiable pattern. However, the results obtained suggested that there was no free acetaldehyde present in the peripheral venous blood of humans metabolizing moderate doses of ethanol and the importance of acetaldehyde in the effects of ethanol in peripheral tissues may be negligible. The estimated levels of acetaldehyde in blood passing to the brain may be sufficient to exert significant pharmacological effects on the brain but further study of the binding of acetaldehyde to tissues is required before a fuller understanding of the toxic potential of acetaldehyde can be gained.

A rat-liver perfusion system, set up to study hepatic ethanol metabolism, was used to determine the nature of hepatic acetaldehyde production. Acetaldehyde production by perfused rat livers was characterized by a peak of acetaldehyde, of variable magnitude, appearing in the hepatic venous perfusate in the first 30 min of the perfusion with medium containing ethanol. After the peak in acetaldehyde production, the metabolism of ethanol by the perfused livers gave negligible amounts of acetaldehyde in the perfusates.

ACKNOWLEDGEMENTS

I wish to thank my supervisors, Professor R.D.Batt and Dr R.M. Greenway for the help they have given me throughout the course of this study. I am also pleased to acknowledge the help and advice received from all the members of the alcohol research group, especially Mr K.Couchman and Dr K.E.Crow. Thanks are also extended to Dr O.Batt for her medical supervision and Mr T.J.Braggins and Mrs K.M.Newland for their expert technical assistance.

CONTENTS

LIST OF FIGURES