ETHANOL AND ACETALDEHYDE METABOLISM

IN SHEEP

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1973
Ethanol and acetaldehyde metabolism in sheep has been studied in three different types of experiments: in purified enzyme systems, in liver homogenates and in the intact animals. Particular emphasis has been placed on the aldehyde oxidase enzyme from sheep liver, a molybdo-flavoprotein with a broad specificity which includes aldehydes, quinines and N1-methyl nicotinamide. This thesis describes a method for preparing an enzyme solution in which sheep liver aldehyde oxidase constitutes 85% of the total protein present. Investigations of its physical and kinetic properties show that the sheep liver enzyme differs from the aldehyde oxidases previously prepared from pig and rabbit livers. In addition, an antibody to sheep liver aldehyde oxidase has been prepared from rabbit serum and has been shown to act as a specific, competitive inhibitor of the enzyme. This has been used to assess the contribution that aldehyde oxidase makes to acetaldehyde oxidation in sheep liver homogenates under several different conditions.

The effects of steroids on ethanol and acetaldehyde metabolism has been investigated, special interest being taken in the effects of progesterone. Progesterone stimulates sheep liver aldehyde oxidase activity \textit{in vitro} and inhibits sheep liver aldehyde dehydrogenase. Administration of progesterone to castrated sheep \textit{in vivo} increased the rates of ethanol and acetaldehyde oxidation, and aldehyde oxidase has been identified as a factor in decreasing acetaldehyde concentrations in the homogenates of livers from these animals during the metabolism of exogenous ethanol. Low endogenous ethanol concentrations in peripheral venous blood of sheep are positively correlated with high progesterone levels in sheep due to its experimental administration, and to pregnancy and the oestrus cycle.

Studies of the effects of disulphiram on ethanol and acetaldehyde metabolism have shown that the compound inhibits sheep liver aldehyde oxidase and aldehyde dehydrogenase enzymes \textit{in vitro}, increases endogenous concentrations of acetaldehyde in peripheral venous blood, and causes acetaldehyde accumulation during ethanol metabolism \textit{in vivo}. When diazepam is present together with disulphiram it provides protection from all but one of the effects shown by disulphiram alone. It does not alter the disulphiram inhibition of sheep liver aldehyde dehydrogenase. Amitriptyline is an inhibitor of both aldehyde oxidase and aldehyde dehydrogenase enzymes. It seems to increase the aldehyde
oxidase response to disulphiram, and its in vivo administration causes acetaldehyde accumulation in peripheral blood during and in the absence of metabolism of exogenous ethanol.

Investigations into the effects of ethanol on ethanol and acetaldehyde metabolism in sheep have shown that ethanol increases the activity of aldehyde oxidase in vitro and its chronic administration accelerates acetaldehyde oxidation in vivo. A supplementary study of the inter-relationships between the relative concentrations of NADH and NAD⁺, and ethanol and acetaldehyde metabolism shows that aldehyde oxidase participation in acetaldehyde oxidation is dependent on the NAD⁺ concentrations, and that acetaldehyde oxidation can account for much of the NADH accumulation that occurs during ethanol metabolism in vivo.

Acetaldehyde oxidation during ethanol metabolism in sheep can be diverted through the aldehyde oxidase catalyzed pathway, avoiding dependence on the NAD⁺-linked aldehyde dehydrogenase enzyme. The results in this thesis have shown that aldehyde oxidase can catalyze up to two-thirds of acetaldehyde oxidation in sheep liver when NAD⁺ is limited, and that the pathway is dependent on the endocrine state and the pattern of ethanol consumption of the animal.
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Upon the advice of Prof. R. D. Batt, this thesis is presented in the form of several papers to facilitate its later publication.
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LIST OF ABBREVIATIONS

a.m. ante meridian
by vol. by volume
CoQ coenzyme Q
D diffusion constant
Δ change in
DOI 2,6-dichlorophenol indophenol
DM dry matter
E.C.No. Enzyme Commission Number
EDTA ethylenediamine-tetra-acetate
FAD flavin adenine dinucleotide
g gravity
HCl hydrochloric acid
K degrees Kelvin
K_i inhibitor constant
K_m Michaelis constant
LD_50 lethal dose for 50% of a population
M molecular weight
MeB methylene blue
mmoles millimoles
mg% mg/100cm^3
N number of samples
NAD^+ nicotinamide adenine dinucleotide
NADH reduced nicotinamide adenine dinucleotide
NADP^+ nicotinamide adenine dinucleotide phosphate
NADPH reduced nicotinamide adenine dinucleotide phosphate
NE not estimated
OD optical density
p relative density
p< probability less than
pH_{max} optimum pH
R universal gas constant
R_f distance travelled relative to the front
RNA ribonucleic acid
s sedimentation coefficient
S Svedburgs
SD standard deviation
\( SE_M \) standard error of the mean

-\( S-S- \) sulphydryl group

tert. tertiary

T absolute temperature

\( \bar{\gamma} \) partial specific volume

vol. volume

w/v weight/volume

v/v by volume

'zero' taken at zero time