chrome P-450 concentration shows the disappearance to be loss of the active conformation of the enzyme rather than inhibition of an enzyme assay, as is employed with most other enzymes. No cytochrome P-450 could be detected by our methods in yeast strains N.C.Y.C. 73, 74, 366, 379, 533, 686, 692 and 716.

A respiratory-deficient mutant of yeast (N.C.Y.C. 240) was shown to produce cytochrome P-450 even in 0.1%-glucose-containing media, where none is normally produced. The mutant tested produced 2.3 nmol of cytochrome P-450/g wet wt. of yeast in 0.1% glucose (and 5.5 nmol in 20% glucose). The mutant does not produce cytochrome $a + a_2$ even in 0.1%-glucose media. The cyclic AMP concentrations here have yet to be investigated.

Effect of hypoglycin on alanine release by skeletal muscles in vitro

DAVID A. DUFF,* SHIRLEY C. PRICE† and KEITH SNEILL*

*Division of Biochemistry and †Division of Toxicology, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

Ingestion of hypoglycin (1,2-amino-3-methylencyclopropylpropionic acid), present in unripe Jamaican ackee fruit, causes a disease characterized by severe hypoglycaemia and disturbances of carbohydrate and lipid metabolism (Sherratt & Osmundsen, 1976; Billington et al., 1978). Hypoglycin is transaminated to methylencyclopropyrolepyruvate, which is oxidatively decarboxylated to methylencyclopropylacetyl-CoA (Kean & Rainford, 1973; Sherratt & Osmundsen, 1976). Methylencyclopropylacetyl-CoA is proposed as the active hypoglycaemic metabolite owing to its inhibition of hepatic gluconeogenesis, largely as a result of changes in acyl-CoA/CoA distributions, which inhibit pyruvate carboxylase activation by acetyl-CoA (Billington et al., 1978; Kean & Pogson, 1979). Hypoglycin or its metabolites inhibit gluconeogenesis in liver and kidney in vitro (Patrick, 1966; Billington, 1976; Kean & Pogson, 1979).

Hypoglycin metabolism is initiated by branched-chain aminotransferase (EC 2.6.1.6). Activity is lowest in liver, but higher in muscle, which, because of its mass, contributes more than 80% of the total body activity (Shinnick & Harper, 1976; Cappuccino et al., 1978). Hypoglycin and its metabolites could therefore owe part of their hypoglycaemic action to effects on the metabolism of muscle. Osmundsen et al. (1978) have shown that hypoglycin in vitro inhibits gluconeogenesis and almost completely abolishes glucose recycling via muscle lactate and alanine. In view of the role of muscle alanine release in the provision of alanine as a gluconeogenic precursor and as recycled glucose carbon (Snell, 1979ab, 1980), the effect of hypoglycin on alanine release by muscle in vitro was studied.

Soleus and extensor digitorum longus muscles of rats (70-90 g) were dissected (Maizels et al., 1977) and incubated (Frayn & Maycock, 1979) in glucose- and albumin-free bicarbonate buffer (Snell & Duff, 1977) for 1 h with or without 3 mM-valine or 3 mM-leucine and 1 mM-hypoglycin. Alanine present in the medium and the tissue at the end of the incubation was measured with alanine dehydrogenase (Williamson, 1974) by fluorimetry. Valine stimulated alanine release from skeletal muscles of fed rats, whereas leucine seemed a poor precursor for alanine formation (Fig. 1). In muscles from starved rats, valine- or leucine-stimulated alanine release was greater than in fed rats (Fig. 1), as with diaphragm muscle (Snell & Duff, 1979). Hypoglycin (1 mM) abolished the amino acid-stimulated release of alanine from muscles of starved rats (Fig. 1). Valine or leucine had no effect on the muscle contents of alanine in any of these experiments.

Since both leucine- and valine-stimulated alanine release was inhibited, it seems that hypoglycin inhibits the provision of amino nitrogen for alanine formation, rather than the pathway for conversion of valine carbon into pyruvate for alanine formation (Snell & Duff, 1977; Snell, 1979). The structural similarity of hypoglycin to branched-chain amino acids and its metabolism by enzymes involved in their oxidation suggest that decreased alanine release may be due to inhibition of branched-chain aminotransferase and a decrease in the formation of glutamate for transamination to alanine. Sequestration of CoA through metabolism of hypoglycin to methylencyclopropylacetyl-CoA has been alternatively implicated in its actions in the liver (Bressler et al., 1969). In muscle, CoA sequestration would inhibit branched-chain oxo acid dehydrogenase, causing an accumulation of branched-chain oxo acids and displacing branched-chain aminotransferase towards valine or leucine at the expense of glutamate.

Regardless of the mechanism for hypoglycin inhibition of branched-chain amino acid metabolism, the decrease in conversion into alanine and in release of muscle alanine may contribute to the hypoglycaemic action of hypoglycin in the whole animal. This would be consistent with the observed abolition of glucose recycling in vivo (Osmundsen et al., 1978). It is noteworthy that in maple-syrup-urine disease (involving a genetic defect in branched-chain oxo acid dehydrogenase) there is a markedly lowered blood concentration of alanine (Haymond et al., 1973), which may account, in part, for the associated hypoglycaemia.

We are grateful to Dr. E. A. Kean for a generous gift of hypoglycin. S. C. P. was supported by an M.R.C. Advanced Course Studentship.
Peripheral substrate exchange in malignant hyperthermia in the pig

GEORGE M. HALL,* JERRY N. LUCKE,† ROGER LOVELL,‡ and DAVID LISTER§

*Department of Anaesthetics, Royal Postgraduate Medical School, London W12 OHS, U.K.; †Department of Veterinary Surgery, University of Bristol, Langford, Bristol BS18 7DU, U.K., and §A.R.C. Meat Research Institute, Langford, Bristol BS18 7DY, U.K.

Malignant hyperthermia is a rare but often fatal complication of general anaesthesia. The syndrome also occurs in certain breeds of pigs, and these have been used extensively as an animal model for the syndrome. The basic defect in malignant hyperthermia is probably an increased Ca²⁺ concentration within the cytoplasm of striated muscle, which causes the characteristic heat production, lactic acidosis and muscle rigidity. Although there have been several studies of changes in circulating metabolites in malignant hyperthermia (Berman et al., 1970; Lucke et al., 1976), it is not possible to determine the exact contribution of the increased muscle metabolism because of the important and variable effect of hepatic metabolism (Hall et al., 1978). In the present study we have systematically examined changes in leg metabolism during the hyperthermic response by using femoral arterial and venous blood samples.

Eight malignant-hyperthermia-susceptible Pietrain pigs were investigated. The pigs were anaesthetized with thiopentone and ventilated with nitrous oxide and oxygen to enable femoral arterial and venous cannulation to be undertaken. Control samples were collected and then malignant hyperthermia was induced by ventilating the pigs with 1% halothane for 10 min together with the intravenous administration of 1 mg of suxamethonium chloride (suxcynicholine chloride)/kg body wt. after 5 min of halothane. Paired femoral arterial and venous samples were collected every 10 min during malignant hyperthermia and analysed for pH, O₂ content, glucose, K⁺, lactate, pyruvate, alanine, non-esterified fatty acids and glycerol. The haematoctrit of the arterial samples was also determined and arterial blood flow estimated by the infusion of the dye Indocyanine green (Jorfeldt & Wahren, 1971).

All the pigs rapidly developed malignant hyperthermia, with an increase in muscle temperature from 38.9 ± 0.1°C (mean ± S.E.M.) in the control period to 42.8 ± 0.3°C after 40 min. An efflux of free glucose from the leg occurred during the first 30 min of the hyperthermic response, indicating massive glycolysis (Wahren, 1970). At 10 min after malignant hyperthermia was induced, lactate production by the leg increased to 0.87 ± 0.29 mmol/min and was associated with a rise in arterial lactate concentration to 8.6 ± 0.6 mmol/litre. A high rate of lactate production continued throughout the hyperthermic response, so that the arterial lactate reached 18.9 ± 1.5 mmol/litre after 40 min, and was accompanied by a small efflux of pyruvate. Although the uptake of lactate by the liver increases during malignant hyperthermia (Hall et al., 1978), it is insufficient to prevent the occurrence of a severe lactic acidosis owing to muscle glycogenolysis. For example, the lactate production by one leg, of 0.65 ± 0.22 mmol/min found in this study after 40 min, is approximately equal to the lactate uptake by the liver measured under identical conditions (Hall et al., 1978). There was a significant decline in the production of non-esterified fatty acids and glycerol during the hyperthermic response, and a small, but insignificant, increase in the efflux of alanine and K⁺. The failure to find an important contribution of skeletal muscle to the marked hyperkalaemia, 7.3 ± 0.6 mmol of K⁺/litre after 10 min, conforms our previous suggestion that the liver is the major site of K⁺ production (Hall et al., 1978).

In spite of the massive increase in muscle metabolism, there was no associated increase in leg blood flow. Indeed, as the hyperthermic response progressed the leg blood flow decreased from 0.40 ± 0.05 litre/min in the control period to 0.25 ± 0.05 litre/min after 40 min. The oxygen consumption by the leg was only slightly above control values, despite maximal oxygen extraction as shown by complete desaturation of the femoral venous blood. Thus in malignant hyperthermia there is a failure of the fundamental homoeostatic mechanism that normally matches an increase in muscle metabolism with a corresponding increase in blood flow. Consequently aerobic metabolism is restricted, so that the Ca²⁺ stimulation of glycogenolysis produces a particularly severe lactic acidosis. It is likely, therefore, that if the factor(s) responsible for the failure of muscle blood flow to increase in malignant hyperthermia can be elucidated, then it will be possible to partially control the severe lactic acidosis.

G. M. H. received generous financial support from the Muscular Dystrophy Group of Great Britain.


Vol. 8