Apparent Reversal of Insulin Resistance in Cardiac Muscle in Alloxan–Diabetes by 2-Bromostearate

INSULIN *in vitro* fails to restore normal rates of glucose uptake, glucolysis and glucose oxidation in the alloxan–diabetic rat heart. This insulin resistance is reversed by hypophysectomy or adrenalectomy, and it may be caused by the inhibition of hexokinase, phosphofructokinase and pyruvate dehydrogenase[1,2]. Similar changes may be induced in hearts from non-diabetic rats by *in vitro* perfusion with fatty acids, so it has been suggested that these changes in the diabetic heart are a consequence of increased rates of hydrolysis of muscle triglyceride and oxidation of fatty acid[2,3]. Attempts to substantiate this suggestion by the use of known antilipolytic drugs were frustrated by their low activity in muscle preparations. The effect of fatty acids may depend on their oxidation leading to elevated tissue concentrations of acetyl Co*A* and citrate which may inhibit pyruvate dehydrogenase, phosphofructokinase and hexokinase[2]. This suggested an alternative approach using inhibitors of fatty acid oxidation and in particular inhibitors of carnitine acyl transferase because of the apparently specific role of this enzyme in fatty acid oxidation. Acyl D-carnitines, which are potent inhibitors of the transferase, were unsuitable because of their toxicity. Tubbs and Chase[4] have described inhibition of carnitine acyl transferase and of fatty acid oxidation in mitochondria with coenzyme *A* derivatives of 2-bromostearate and 2-bromolaurate. Conditions have been defined in which 2-bromostearate can be used as an inhibitor of fatty acid oxidation in the perfused heart, and these results are described here.

The analytical methods used and the technique for perfusion with long chain fatty acids have been described before[2,5]. Preliminary experiments showed that perfusion could be made with 2-bromostearate in combination with bovine plasma albumin; that maximum effects on fatty acid and glucose metabolism required perfusion for 10 min with 1 mM 2-bromostearate and 2 per cent albumin; and that these effects persisted when perfusion was continued with medium free of bromostearate. By visual inspection the rate and force of contraction of diabetic hearts were maintained for 12–16 min with bromostearate. Contractions then slowed and became weaker and ceased after 18–20 min. In normal hearts the periods were shorter (9–11 and 11–14 min). The technique adopted was to perfuse by recycling for 10 min with medium containing 2 per cent albumin, 1 mM 2-bromostearate, 100 mg/100 ml. glucose, and 0·05 U/ml. insulin; and then to continue perfusion with glucose and insulin medium only. In these conditions reasonably normal beating was maintained for at least 30 min.

The results are given in Table 1. The diabetic heart showed the expected diminutions in the rates of glucose uptake, glucolysis and glucose oxidation; and inhibition of phosphofructokinase and hexokinase through citrate accumulation as shown by the changes in tissue concentrations of citrate, hexose phosphates and intracellular glucose. These changes were apparently completely reversed by 2-bromostearate without any concomitant alteration in adenine nucleotide or glycogen concentrations. With the possible exception of a further increase in the rate of conversion of U–^{14}C glucose to ^{14}C carbon dioxide, normal hearts showed no change in these parameters with 2-bromostearate (not shown). Glucose oxidation accounted for 23 per cent of the oxygen consumption in the diabetic heart and this increased to 77 per cent with bromostearate. It has been shown[3] that the balance is a consequence of fatty acid oxidation, so it can be calculated that bromostearate inhibited fatty acid oxidation by 70 per cent in the diabetic tissue. In a further experiment (not shown) in which perfusions were made with 5 mM pyruvate, 2-bromostearate increased the uptake of pyruvate by 74 per cent and its oxidation almost six-fold. Subject to the proviso that the mechanism of the bromostearate effect needs to be established in the tissue, these findings indicate that an inhibitor of fatty acid oxidation can reverse these abnormalities of glucose and pyruvate metabolism in the diabetic tissue.

Table 1. EFFECT OF 2-BROMOSTEARATE ON GLUCOSE METABOLISM AND METABOLITE CONCENTRATIONS IN ALLOXAN–DIABETIC RAT HEART

Parameter	Mean ± S.E. for Alloxan–Diabetic Control	Alloxan–Diabetic 2-Bromostearate	Normal control
Rate: μmoles/g/dry ventricle/h			
Glucose uptake	140 ± 17	325 ± 37	318 ± 17
Glucolysis	140 ± 17	325 ± 37	318 ± 17
Glucose oxidation*	54 ± 16	192 ± 37	226 ± 13
Rate d.p.m. in CO_2/g dry ventricle/5 min			
Glucose oxidation	3,500 ± 1,720	23,200 ± 4,430	17,200 ± 2,380
Concentration (mM)			
Intracellular glucose	1·61 ± 0·12	1·11 ± 0·10	0·87 ± 0·13
Concentration: μmoles/g/dry ventricle			
Glucose 6-phosphate	3·05 ± 0·09	1·68 ± 0·09	1·66 ± 0·08
Fructose 6-phosphate	0·93 ± 0·11	0·61 ± 0·04	—
Fructose 1 : 6-diphosphate	0·033 ± 0·008	0·16 ± 0·03	0·12 ± 0·013
Citrate	2·85 ± 0·16	0·72 ± 0·09	0·98 ± 0·13
ATP	18·1 ± 0·50	19·2 ± 0·48	19·1 ± 0·48
ADP	3·4 ± 0·34	3·3 ± 0·25	—
AMP	0·9 ± 0·12	0·8 ± 0·09	—
Glycogen			
10 min preperfusion	229 ± 26	194 ± 21	—
10 min + 20 min perfusion	252 ± 14	200 ± 30	—

Hearts were preperfused for 10 min with medium containing either 2 per cent bovine plasma albumin (controls) or 2 per cent albumin + mM 2-bromostearate, with glucose (100 mg/100 ml.) and insulin (0·05 U/ml.) and then perfused for a further 10 or 20 min with medium containing glucose and insulin alone. Glucose oxidation rates were calculated either as (glucose uptake–lactate output–glycogen synthesis rates)* or as d.p.m. in CO_2 after perfusion with medium containing U–^{14}C glucose (10,000 d.p.m./μmole). Glycogen was estimated after 10 min of preperfusion and also after a further 20 min of perfusion. The minimum number of hearts perfused in any one group was six.

Burges *et al.*[6] have recently described in a preliminary report increased rates of glucose uptake and glucose oxidation in the diabetic heart with 2-bromopalmitate. Complete restoration of normal rates may not have been achieved in their experiments and this quantitative difference could be explained by the preperfusion technique used in the present studies. 2-Bromo fatty acids may thus be a useful tool for establishing the role of fatty acid oxidation in metabolic disturbances in experimental diabetes.

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220 Mc Nuclear Magnetic Resonance Spectra of Gramicidin *S* in Solution*

IN a previous communication[1] we reported the nuclear magnetic resonance (NMR) spectra at 100 Mc of gramicidin *S* in dimethylsulphoxide (DMSO) solution and made

* Readers should know that Bradbury *et al.* (*Nature*, **220**, 69; 1968) have shown that the attempt to interpret chemical shifts in terms of conformation is almost certainly futile.