Lipoamide influences substrate selection in post-ischaemic perfused rat hearts

Balazs SUMEGI,*†|| Nina B. BUTWELL,‡§ Craig R. MALLOY‡§ and A. Dean SHERRY†§

*University Medical School Pecs, Department of Biochemistry, Szegedi út 12, 7624-Pecs, Hungary, †Department of Chemistry, University of Texas at Dallas, P.O. Box 830688, Richardson, TX 75083-0688, U.S.A., and ‡Department of Internal Medicine (Cardiology Division) and §The Ralph B. and Mary Nell Rogers Biomedical Magnetic Resonance Center, Department of Radiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235, U.S.A.

We investigated whether lipoamide and diacetyl-lipoamide are able to change the substrate selection in post-ischaemic myocardium. This can be important, because shifting heart metabolism from fatty acid to carbohydrate oxidation can decrease ischaemic injury. Studying the metabolism of [1,2-¹³C]diacetyl-lipoamide *in situ* in perfused rat heart by ¹³C n.m.r., we found intense ¹³C labelling in glutamate and aspartate, showing that acetyl groups from diacetyl-lipoamide are effectively transferred to CoA and metabolized in heart tissue. From analysis of glutamate C-3 and C-4 isotopomers, we determined the [1,2-¹³C]acetate/[3-¹³C]lactate utilization ratio in normoxic and post-ischaemic hearts, where under our experimental conditions the acetate/lactate utilization ratios were 1.2 ± 0.2 and 2.4 ± 0.3 in normoxic and post-ischaemic hearts respectively. When 0.25 mM

lipoamide was added to the perfusate the acetate/lactate utilization ratio decreased to 1.4 ± 0.1 , which is almost equal to that found for normoxic hearts, showing that lipoamide increased the lactate utilization. In accordance with these data, we found that lipoamide activated pyruvate dehydrogenase by 50% in post-ischaemic myocardium. Competition between [3-¹³C]lactate and unlabelled octanoate was also studied in post-ischaemic hearts, and we found that lipoamide increased lactate utilization by 100% and increased the rate of the tricarboxylic acid cycle by 64%. Under the same experimental conditions, lipoamide significantly promoted the recovery of post-ischaemic unperfused hearts, showing the positive effect of increased lactate oxidation in post-ischaemic myocardium.

INTRODUCTION

A variety of endogenous substrates can contribute to the pool of acetyl-CoA entering the tricarboxylic acid cycle in heart tissue. Although some features of substrate selection are well established [1–6], the relative utilization of all substrates available to the heart under various physiological conditions may be quite subtle and complex. Ischaemia influences substrate selection in perfused hearts [4,7], and these changes could affect recovery of post-ischaemic hearts. Therefore it is important to design new methods which might make it possible to influence substrate selection in post-ischaemic myocardium. Previous experiments have suggested that a high acetyl-CoA/CoA ratio in post-ischaemic hearts may be responsible for many metabolic abnormalities [8,9], and it has been suggested that carnitine may help to alleviate this problem by acting as a general acyl-group acceptor from acyl-CoAs, thereby decreasing the acetyl-CoA/CoA ratio [10,11]. However, a high carnitine concentration may increase the concentrations of long-chain acyl-CoA and acyl-carnitine [10], which have an inhibitory effect on the mitochondrial adenine nucleotide transporter [12]. Therefore it could be advantageous to identify substrates which decrease the acetyl-CoA/CoA ratio without causing the known deleterious effects of increasing the levels of long-chain acyl-CoAs within mitochondria. Lipoic acid (which acts as a cofactor when covalently bound to lysine residues in the second component of the α -oxo-acid dehydrogenase complexes), is known to act as an acceptor of short-chain acyl groups from acyl-CoA *in vitro*. Other data have shown that lipoamide and some lipoamide derivatives are even better cofactors for the α -oxo-acid dehydrogenase reactions than is free

lipoic acid [13]. These small molecules can participate in the reactions in Scheme 1.

Scheme 1 Possible involvement of lipoamide in mitochondrial enzyme reactions

Abbreviations: Lip DH, lipoamide dehydrogenase; PDC, pyruvate dehydrogenase complex; OGDC, α -oxoglutarate dehydrogenase complex.

These reactions suggest that lipoamide may provide a mechanism for decreasing the acetyl-CoA/CoA ratio in post-ischaemic heart without increasing the concentration of long-chain acyl-CoA.

We have previously reported ¹³C n.m.r. methods [14–17] for measurement of substrate utilization in the intact myocardium during both steady-state and non-steady-state conditions. In this paper we have used these techniques to examine metabolism of [1,2-¹³C]diacetyl-lipoamide in normal and post-ischaemic hearts, and to examine the effect of free lipoamide on the substrate selection by post-ischaemic myocardium.

MATERIALS AND METHODS

Materials

Sodium [2-¹³C]acetate, [3-¹³C]acetate, [1,2-¹³C]acetyl chloride and [3-¹³C]pyruvate were obtained from MDS Isotopes (St. Louis, MO, U.S.A.). DL-Lipoamide was obtained from Sigma. All other

|| To whom correspondence should be addressed, in Hungary.

chemicals were of the highest purity commercially available. Dihydrolipoamide was prepared from DL-lipoamide as described [18] and acetylated by reaction with [1,2- ^{13}C]acetyl chloride (10% excess) in chloroform/triethylamine (4:1, v/v). After removal of the solvent by low-pressure evaporation, the remaining oil was extracted into dry chloroform, insoluble materials were removed by filtration, and the solvent was once again removed by evaporation. This same extraction procedure was repeated with diethyl ether, and the purity of [1,2- ^{13}C]diacetyl-lipoamide was determined by ^{13}C n.m.r. This product gave an n.m.r. spectrum identical with that of a product previously synthesized by enzymic methods [19].

Heart perfusions

Male Sprague-Dawley rats weighing 300–350 g were anaesthetized in an ether atmosphere. Hearts were rapidly excised and placed briefly in a 4 °C arrest medium. The aorta was immediately cannulated, and hearts were perfused retrogradely at a pressure of 70 cmH₂O. A modified phosphate-free Krebs-Henseleit perfusate was used, which contained 118 mM NaCl, 5.0 mM KCl, 1.25 mM CaCl₂, 1.2 mM MgSO₄ and 25 mM NaHCO₃, and was gassed with O₂/CO₂ (19:1) and maintained at 37 °C. A series of hearts were perfused with one of the following substrate combinations to establish baseline metabolic profile: (a) 0.5 mM [1,2- ^{13}C]diacetyl-lipoamide; (b) 0.5 mM [1,2- ^{13}C]diacetyl-lipoamide plus 2 mM [3- ^{13}C]pyruvate; and (c) 0.25 mM [1,2- ^{13}C]acetate plus 1 mM [3- ^{13}C]lactate. After 10 min of normoxic perfusion, the hearts were freeze-clamped.

Four additional groups of hearts were perfused to determine the effect of lipoamide on the substrate selection in heart: (a) 15 min global ischaemia (no added substrates before and under ischaemia), 10 min perfusion with 0.25 mM [1,2- ^{13}C]acetate plus 1 mM [3- ^{13}C]lactate; (b) 15 min global ischaemia (no added substrates before and under ischaemia), 10 min perfusion with 0.25 mM [1,2- ^{13}C]acetate, 1 mM [3- ^{13}C]lactate plus 0.25 mM lipoamide; (c) 10 min perfusion in the presence of 2 mM sodium octanoate before ischaemia, followed by 15 min global ischaemia and 10 min perfusion with 2 mM unlabelled sodium octanoate plus 2 mM [3- ^{13}C]lactate; (d) 10 min perfusion in the presence of 2 mM sodium octanoate before ischaemia, followed by 15 min global ischaemia and 10 min perfusion with 2 mM unlabelled sodium octanoate, 2 mM [3- ^{13}C]lactate plus 0.4 mM lipoamide. At the end of the perfusion hearts were freeze-clamped. All frozen heart tissue was extracted with 7% HClO₄, neutralized with KOH, freeze-dried, and redissolved in $^2\text{H}_2\text{O}$ for n.m.r.

N.m.r. methods

Proton-decoupled ^{13}C n.m.r. spectra were obtained at 125.7 MHz on a GN 500 spectrometer by using a 45° observe pulse, an interpulse delay of 4.5 s, and bilevel proton decoupling with WALTZ. Proton spectra were recorded on the same samples by using a 5 s pre-saturation pulse to saturate the HO ^2H resonance.

Enzyme assays

The extractable pyruvate dehydrogenase activity was determined as described in [20], and citrate synthase activity was determined as described in [21].

RESULTS

[1,2- ^{13}C]Diacetyl-lipoamide and [3- ^{13}C]pyruvate perfusions

A ^{13}C spectrum of a heart perfused for 10 min with [1,2- ^{13}C]diacetyl-lipoamide as the only external substrate is shown in

Figure 1(a). The multiplets observed in each glutamate resonance reflect a highly ^{13}C -enriched acetyl-CoA pool in this heart, showing that [1,2- ^{13}C]diacetyl-lipoamide can act as an efficient acetyl-group donor. A ^{13}C isotopomer analysis of the glutamate spectrum [22] indicated that [1,2- ^{13}C]diacetyl-lipoamide provided about 75% of the total acetyl-CoA utilized by this heart during the 10 min perfusion period. To determine whether pyruvate can compete effectively with [1,2- ^{13}C]diacetyl-lipoamide as source of acetyl groups, another group of hearts was perfused with a mixture of 0.5 mM [1,2- ^{13}C]diacetyl-lipoamide and 2 mM [3- ^{13}C]pyruvate. The n.m.r. spectrum of one such heart extract is shown in Figure 1(b). A ^{13}C isotopomer analysis [15,22] of this spectrum indicates that [1,2- ^{13}C]diacetyl-lipoamide provided 45% of the total acetyl-CoA, whereas [3- ^{13}C]pyruvate provided 34% of the total acetyl-CoA which entered the tricarboxylic acid cycle in this heart. Thus, even in the presence of a 4-fold excess of pyruvate, the acetyl groups of diacetyl-lipoamide are better utilized by heart tissue.

Effect of free lipoamide on metabolism of other competing ^{13}C -enriched substrates

When a mixture of 0.25 mM [1,2- ^{13}C]acetate and 1 mM [3- ^{13}C]lactate is given to a perfused heart, each substrate contributes about equally to the acetyl-CoA pool [17]. Typical high-res-

Figure 1 ^{13}C n.m.r. spectrum of rat hearts perfused with (a) 0.5 mM [1,2- ^{13}C]diacetyl-lipoamide and (b) 0.5 mM [1,2- ^{13}C]diacetyl-lipoamide plus 2 mM [3- ^{13}C]pyruvate

Hearts were perfused for 10 min in the presence of ^{13}C -enriched substrate(s). All other conditions were as described in the Materials and methods section. Abbreviations used: T, taurine C-2 and C-3; G2, G3 and G4, glutamate C-2, C-3 and C-4 respectively; A2 and A3, aspartate C-2 and C-3 respectively; C2,4, citrate C-2 and C-4.

Figure 2 ^{13}C n.m.r. spectra of normoxic (a), post-ischaemic (b) and post-ischaemic plus 0.25 mM lipoamide (c)-treated hearts

Rat hearts were perfused with 1 mM $[3-^{13}\text{C}]$ lactate plus 0.25 mM $[1,2-^{13}\text{C}]$ acetate for 10 min (a), or the perfusion with ^{13}C -labelled substrates took place after a 15 min period of global ischaemia (b and c). All other conditions were as described in the Materials and methods section. Abbreviations used: T, taurine C-2 and C-3; G2, G3 and G4, glutamate C-2, C-3 and C-4 respectively.

olution ^{13}C n.m.r. spectra of extracts of such hearts are presented in Figure 2. As shown previously [17], the area of the singlet + C-3–C-4 doublet versus the area of the C-4–C-5 doublet + quartet in the glutamate C-4 resonance directly reports the relative utilization of $[3-^{13}\text{C}]$ lactate versus $[1,2-^{13}\text{C}]$ acetate. Thus the larger singlet component in the glutamate C-4 resonance (34 p.p.m.) of the normoxic heart compared with the ischaemic heart (compare resonance at 34 p.p.m. in Figures 2a and 2b) allows one to conclude that oxidation of $[3-^{13}\text{C}]$ lactate is inhibited in ischaemic tissue. The isotopomer analysis of the data in Figure 2 shows that the acetate/lactate utilization ratio is 1.2 ± 0.2 in normoxic hearts and about 2.4 ± 0.3 in hearts reperfused for 10 min after 15 min of global ischaemia, which is in accord with our previous data [17]. We have also shown that the acetate/lactate utilization ratio returns to control values after longer reperfusion periods (30–40 min), and attributed this to reactivation of the pyruvate dehydrogenase complex during reperfusion. The ^{13}C n.m.r. spectrum of an ischaemic heart reperfused for 10 min in the presence of 0.25 mM lipoamide plus the same combination of

Table 1 Effect of lipoamide on pyruvate dehydrogenase activity in perfused rat heart

Experimental conditions are the same as described in Figure 2. Results are means ($n = 3$): * $P = 0.12$, and ** $P = 0.02$, compared with control group.

Treatment of heart	Activity of pyruvate dehydrogenase (m-units/mg of protein)	
	No added lipoamide	240 μM lipoamide
Normoxic	24.4 ± 2.8	$29.2 \pm 3.2^*$
Post-ischaemic	20.9 ± 2.9	$30.8 \pm 3.4^{**}$

labelled substrates as described above is shown in Figure 2(c). The glutamate C-4 resonance in this spectrum resembles more the spectrum for a normoxic heart (Figure 2a) than that of an ischaemic heart (Figure 2b). It indicates that lipoamide alters the relative utilization of acetate and lactate in ischaemic heart tissue. Quantitative results for a series of hearts reperfused with 0.25 mM lipoamide indicates that the acetate/lactate utilization ratio is 1.4 ± 0.1 , nearly identical with the ratio found for normoxic hearts.

These results suggested that lipoamide may alter the activity of pyruvate dehydrogenase in post-ischaemic heart. To evaluate this question further, we measured the actual pyruvate dehydrogenase activity in normoxic and post-ischaemic hearts, with and without lipoamide (Table 1). In normoxic hearts, the presence of lipoamide resulted in only a small increase in pyruvate dehydrogenase activity, but this difference in activity was not statistically significant. When lipoamide was added to post-ischaemic hearts, the actual pyruvate dehydrogenase activity increased significantly by about 50% (Table 1). These data indicate that lipoamide promotes lactate oxidation in post-ischaemic myocardium by activation of the pyruvate dehydrogenase complex.

We have also studied if lipoamide has any effect on functional recovery of hearts after short periods of ischaemia. Two groups of rat hearts were perfused with 2 mM octanoic acid to maximize the levels of acyl-CoAs and acetyl-CoA. After global ischaemia for 15 min, they were reperfused with 2 mM octanoic acid plus 2 mM $[3-^{13}\text{C}]$ lactate in the absence or presence of 0.4 mM lipoamide. When four ischaemic hearts were reperfused in the absence of lipoamide, no recovery was seen (neither heart-beat nor pressure was detectable). However, the addition of lipoamide to hearts after 15 min of ischaemia resulted in the recovery of four hearts from five. In those four cases the pressure developed was between 55 and 100% of the original, but the recovered hearts functioned at substantially lower rates than the normal hearts. When these same experiments were performed on paced hearts, both control and lipoamide-treated hearts recovered completely (results not shown).

Since the 2 mM octanoate contributed to a substantial amount of unlabelled acetyl-CoA in these hearts, we could not determine the contribution of $[3-^{13}\text{C}]$ lactate to the acetyl-CoA pool, using usual isotopomer procedures [22], because the ^{13}C resonances of all metabolites appeared as singlets (Figure 3). However, the relative amount of ^{13}C incorporated into the glutamate C-4 position was determined by comparing the area of the glutamate C-4 resonance with the average areas of the two naturally abundant taurine resonances at 48 and 36.5 p.p.m. (Table 2). We also determined the relative taurine and glutamate concentrations in all samples by proton n.m.r., and these data indicated that

Figure 3 Effect of lipoamide on the oxidation of [3-¹³C]lactate in post-ischaemic rat hearts

Rat hearts were perfused with 2 mM sodium octanoate, subjected to a 15 min period of global ischaemia, and then reperfused with 2 mM sodium octanoate and 2 mM [3-¹³C]lactate (a), or with this same substrate combination plus 0.4 mM lipoamide (b). The ¹³C appearing in the glutamate carbon pools was derived from [3-¹³C]lactate; the taurine resonances reflect naturally abundant levels of ¹³C. Abbreviations used: T, taurine C-2 and C-3; G2, G3 and G4, glutamate C-2, C-3 and C-4 respectively.

Table 2 Effect of lipoamide on [3-¹³C]lactate metabolism in post-ischaemic rat heart

Rat hearts were perfused with 2 mM sodium octanoate for 10 min, and subjected to 15 min of global ischaemia (by stopping the flow of perfusate), then reperfused for 10 min with a new perfusate containing 2 mM sodium octanoate and 2 mM [3-¹³C]lactate (control), or with the same substrate combination plus 0.4 mM lipoamide. All other conditions were as described in the Materials and methods section: **P* = 0.005 compared to control group.

	Control (<i>n</i> = 4)	Added lipoamide (0.4 mM) (<i>n</i> = 5)
Total area of glutamate C-4 resonance relative to taurine	0.29 ± 0.08	0.61 ± 0.06*
G4/G3	0.56 ± 0.02	0.92 ± 0.1*

neither metabolite concentration changed significantly in any of the heart extracts examined (with or without lipoamide) (results not shown). The data in Table 2 indicate that lipoamide effectively doubles the amount of ¹³C incorporated into the glutamate C-4 from [3-¹³C]lactate. This can be the consequence of an increased pyruvate dehydrogenase activity, as indicated above, or an increased tricarboxylic acid cycle flux during the 10 min reperfusion period in hearts treated with lipoamide. The glutamate C-4/C-3 ratio (or C-4/C-2 ratio) can be used as an index of tricarboxylic acid cycle flux, as recently suggested [23]; the increase observed in the C-4/C-3 ratio suggests that lipoamide

also increased the rate of the tricarboxylic acid cycle in post-ischaemic hearts by about 50 % (Table 2).

DISCUSSION

Our data indicate that diacetyl-lipoamide is an excellent substrate for the perfused rat heart, even though there is no evidence for a transport system for lipoamide derivatives. The only known reaction for diacetyl-lipoamide is catalysed by the second component of the pyruvate dehydrogenase complex [13,24], and diacetyl-lipoamide proved to be an even better acetyl donor than pyruvate in perfused hearts. Our observation that 0.5 mM diacetyl-lipoamide contributed 45 % of acetyl groups entering the acetyl-CoA pool in the same heart where 2 mM pyruvate contributed only 34 % of the acetyl groups indicated that lipoamide derivatives might be excellent tools for modifying mitochondrial metabolism in heart during conditions when metabolite levels are dramatically altered, such as in ischaemic myocardium.

Our data show that lipoamide promotes oxidation of lactate rather than acetate or fatty acids in the post-ischaemic myocardium and also improves functional recovery in unperfused hearts. Lipoamide may alter the cellular metabolism of lactate (or other glucogenic sources) via at least two mechanisms. First, lipoamide can act as an acetyl-group acceptor, thereby decreasing the immediate deleterious effects of a high concentration of acetyl-CoA in reperfused ischaemic tissue. The best-known regulatory effects of high acetyl-CoA/CoA ratio in the mitochondria are the product inhibition of the pyruvate dehydrogenase complex [13,24] and the activation of pyruvate dehydrogenase kinase, which phosphorylates and thereby inactivates the pyruvate dehydrogenase complex [25]. In addition, the very low free CoA concentration may also inhibit α -oxoglutarate oxidation [24,26] and so the tricarboxylic acid cycle which is the main consumer of acetyl-CoA.

In ischaemic heart the pyruvate dehydrogenase complex is inactivated by covalent modification, which cannot be reversed as quickly as product inhibition, and the low substrate availability, which are the main regulatory factors for acetate and fatty acid metabolism [27]. Therefore, in the first 10–15 min of reperfusion the substrate selection of post-ischaemic hearts are shifted, and acetate and/or fatty acids become the major carbon source for mitochondrial energy production. The dominant fatty acid oxidation and the high concentration of long-chain acyl-CoAs in post-ischaemic hearts have a negative effect on the recovery [4,7]; therefore it is advantageous to shift back the substrate selection of post-ischaemic myocardium from fatty acids to carbohydrate.

The main metabolic effects of lipoamide [a, the activation of the pyruvate dehydrogenase complex (Table 1); b, the increased pyruvate oxidation (Table 2) and activation of the tricarboxylic acid cycle (Table 2)] are probably the consequence of the decreased acetyl-CoA/CoA ratio in the mitochondria. Activation of the tricarboxylic acid cycle can be extremely important, because the higher rate of the cycle can regenerate the free CoA from acetyl-CoA and thereby re-establish a normal acetyl-CoA/CoA ratio in post-ischaemic myocardium. There is evidence showing that carnitine can have a protective effect on the ischaemic myocardium and aid in the recovery of post-ischaemic hearts [10,11]. As carnitine is also known to be an excellent acyl acceptor, the mechanism of its protective effect may be similar to that of lipoamide, i.e. by increasing the level of free CoA [10,11]. The advantage of lipoamide over carnitine is that lipoamide cannot promote transport of fatty acids into mitochondria. Lipoamide may also have a substantial advantage over the

natural vitamin, lipoic acid, because lipoamide is a better substrate of lipoamide dehydrogenase, and dihydrolipoamide is a much better substrate for the second component of α -oxo-acid dehydrogenase complexes than is dihydrolipoic acid [13,24]. Finally, dihydrolipoamide can also act as an antioxidant, by entrapping free radicals formed during lipid peroxidation, which may contribute to its cardioprotective effect, as it was suggested for dihydrolipoic acid [28].

Support for this research was given by N.I.H. grant HL34557 (A.D.S.) and grants OTKA T5057, T006018, T006360 and T2010 from the Hungarian Science Foundation (B.S.).

REFERENCES

- Drake, A. J., Haines, J. R. and Noble, M. I. M. (1980) *Cardiovasc. Res.* **14**, 65–72
- Williamson, J. R. (1962) *Biochem. J.* **83**, 377–383
- Neurohr, K. J., Barrett, E. J. and Shulman, R. G. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1603–1607
- Saddik, M. and Lopaschuk, G. D. (1991) *J. Biol. Chem.* **266**, 8162–8170
- Newsholme, E. A., Randle, P. J. and Manchester, K. S. (1962) *Nature (London)* **193**, 270–271
- Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Pask, H. T. and Denton, R. M. (1976) *Biochem. J.* **154**, 327–348
- Lopaschuk, G. D., Spafford, M. A., Davies, N. J. and Wall, S. R. (1990) *Circ. Res.* **66**, 546–553
- Oram, J. F., Bennetch, S. L. and Neely, J. R. (1973) *J. Biol. Chem.* **248**, 5299–5309
- Folts, J. D., Shug, A. L., Koke, J. R. and Bittar, N. (1978) *Am. J. Cardiol.* **41**, 1209–1214
- Broderick, T. L., Quinney, H. A. and Lopaschuk, G. D. (1992) *J. Biol. Chem.* **267**, 3758–3763
- Paulson, D. J. and Shug, A. L. (1982) *Basic Res. Cardiol.* **77**, 460–463
- Shug, A. L., Thomsen, J. H., Folts, J. D., Bittar, J., Klein, M. I., Koke, J. R. and Huth, P. J. (1978) *Arch. Biochem. Biophys.* **187**, 25–33
- Hucho, F. (1975) *Angew. Chem. Int. Ed. Engl.* **14**, 594–601
- Malloy, C. R., Sherry, A. D. and Jeffrey, F. M. H. (1988) *J. Biol. Chem.* **263**, 6964–6971
- Malloy, C. R., Thompson, J. R., Jeffrey, F. M. H. and Sherry, A. D. (1990) *Biochemistry* **29**, 6756–6761
- Sherry, A. D., Malloy, C. R., Roby, R. E., Rajagopal, A. and Jeffrey, F. M. H. (1988) *Biochem. J.* **254**, 593–598
- Sherry, A. D., Malloy, C. R., Zhao, P. and Thompson, J. R. (1992) *Biochemistry* **31**, 4833–4837
- Reed, L. J., Koike, M., Levitch, M. E. and Leach, F. R. (1958) *J. Biol. Chem.* **232**, 143–158
- O'Connor, T., Roche, T. E. and Paukstelis, J. V. (1982) *J. Biol. Chem.* **257**, 3110–3112
- Hinman, L. M. and Blass, J. P. (1981) *J. Biol. Chem.* **256**, 6583–6586
- Srere, P. A., Brazil, H. and Gonen, L. (1963) *Acta Chem. Scand.* **17**, S129–S134
- Malloy, C. R., Sherry, A. D. and Jeffrey, F. M. H. (1990) *Am. J. Physiol.* **259**, H987–H995
- Fitzpatrick, S. M., Hetherington, H. P., Behar, K. L. and Shulman, R. G. (1990) *J. Cereb. Blood Flow Metab.* **10**, 170–179
- Reed, L. J. (1974) *Acc. Chem. Res.* **7**, 40–46
- Cate, R. L. and Roche, T. E. (1978) *J. Biol. Chem.* **253**, 496–503
- Yeaman, S. J. (1989) *Biochem. J.* **257**, 625–632
- Schulz, H. (1991) *Biochim. Biophys. Acta* **1081**, 109–120
- Suzuki, Y. J., Tsuchiya, M. and Packer, L. (1991) *Free Radical Res. Commun.* **15**, 255–263