Regulation of glycine catabolism in rat liver mitochondria

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1. The catabolism of glycine was studied in isolated rat liver mitochondria by measuring release of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ -glycine. Incubation of mitochondria in a medium containing $0.5\ \mu\text{M}$ free Ca^{2+} resulted in an 8-fold increase in the rate of degradation of glycine. Intraperitoneal injection of glucagon (33 or $100\ \mu\text{g}/100\ \text{g}$ body wt.) 25 min before killing of rats also resulted in a 3-fold or 10-fold (depending on dosage) increase in the rate of catabolism of glycine. 2. Both the stimulation by free Ca^{2+} and that by injection of glucagon *in vivo* were dependent on phosphate in the incubation medium. This requirement for phosphate was specific, as replacement of phosphate by other permeant anions such as thiocyanate and acetate did not permit the stimulation. The phosphate-dependent stimulation of glycine catabolism by Ca^{2+} was also evident when mitochondria were incubated in the absence of K^+ . 3. Mitochondria isolated from rats previously injected with glucagon showed elevated rates of degradation of glycine even in the presence of rotenone, provided that regeneration of NAD^+ was effected by providing acetoacetate. 4. Hypo-osmolality of the medium markedly stimulated the rate of degradation of glycine by mitochondria. Although hypo-osmolality-induced stimulation of glycine degradation was accompanied by parallel changes in mitochondrial matrix volume, no measurable changes in matrix volume were observed in mitochondria stimulated either by free Ca^{2+} ($0.5\ \mu\text{M}$) or by injection of glucagon *in vivo*. Furthermore, Ca^{2+} stimulated glycine decarboxylation in mitochondria exposed to either hyper-osmolar (410 mosmol) or hypo-osmolar (210 mosmol) conditions. Although hyper-osmolality decreased and hypo-osmolality increased matrix volume, stimulation of glycine degradation by Ca^{2+} was not associated with any further changes in matrix volume. 5. These data demonstrate that the regulation of hepatic glycine oxidation by glucagon and by free Ca^{2+} is largely independent of changes in mitochondrial matrix volume.

INTRODUCTION

Catabolism of glycine occurs principally in the liver, which carries out the following reaction through the glycine-cleavage enzyme system (GCS), also known as glycine synthase (EC 2.1.2.10):

Glycine + NAD^+ + tetrahydrofolate $\rightarrow$

CO_2 + NH_3 + N^5N^{10} -methylene tetrahydrofolate + $\text{NADH} + \text{H}^+$

GCS consists of four enzyme proteins present as an enzyme complex in the mitochondrial inner membrane (Kikuchi, 1973). Although much is known about the structure and mechanism of action of GCS (Kikuchi, 1973) only a few studies have attempted to explain the physiological regulation of GCS. We have recently shown that hepatic glycine catabolism is hormonally controlled (Jois *et al.*, 1989) and the mechanism of regulation is different from the previously reported mechanisms of regulation of GCS by branched-chain oxo acids (O'Brien, 1978; Kochi *et al.*, 1986) and by the oxidation–reduction state of mitochondrial nicotinamide nucleotides (Hampson *et al.*, 1983, 1984). Glucagon, catecholamines and vasopressin all stimulate glycine catabolism (Jois *et al.*, 1989, 1990a). An important consideration is how the hormonal signals are transmitted to the mitochondria. In this connection it appears that Ca^{2+} may play an important role. It is known that all these hormones cause an increase in cytosolic Ca^{2+} and we have shown that sub-micromolar concentrations of Ca^{2+} stimulate flux through GCS in isolated rat liver mitochondria (Jois *et al.*, 1990a). In the present paper, we report further studies on the regulation of flux through GCS in isolated mitochondria.

MATERIALS AND METHODS

Materials

$[1-^{14}\text{C}]$ Glycine was obtained from DuPont–New England Nuclear. Sodium acetoacetate was prepared from hydrolysis of ethyl acetoacetate at room temperature for 3 h by 10% molar excess of NaOH , followed by neutralization with HCl and freeze-drying. All other reagents were of analytical grade.

Preparation and incubation of mitochondria

Mitochondria were prepared from livers of male Sprague–Dawley rats weighing 200–250 g. Rats were killed by cervical dislocation, and livers were removed immediately and homogenized in ice-cold isolation medium containing 0.225 M-mannitol, 0.075 M-sucrose, 5 mM-Hepes and 1 mM-EGTA (pH 7.2). Mitochondria were isolated as described previously (Jois *et al.*, 1990a). The final mitochondrial pellet was resuspended in isolation medium at about 30–40 mg of protein/ml for studies on measurement of flux through GCS and at about 80 mg/ml for studies on measurement of mitochondrial matrix volume. Mitochondrial protein was measured by the biuret procedure (Gornall *et al.*, 1949), with BSA as standard. Flux through GCS was determined in an incubation medium consisting of, unless otherwise stated, 125 mM-KCl, 10 mM-Mops, 7 mM-Tris base, 2.5 mM-potassium phosphate, 2.5 mM- MgCl_2 , 5 mM- NaCl , 1 mM-ADP, 5 mM- $[1-^{14}\text{C}]$ glycine and 1 mM-EGTA. When required, medium also contained 0.8 mM- CaCl_2 to give a free Ca^{2+} concentration of $0.5\ \mu\text{M}$. In some experiments the concentration of KCl was adjusted to vary osmolality of the medium. Mitochondria were incubated at 30°C for 15 min. Incubations were terminated with 0.3 ml of 30% (v/v) HClO_4 , and $^{14}\text{CO}_2$ was

Abbreviation used: GCS, glycine-cleavage system.

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collected and counted for radioactivity as previously described (Jois *et al.*, 1989). The preparation of stock and working solutions of EGTA and CaCl_2 was as described by Denton *et al.* (1978).

Mitochondrial matrix volume was measured in mitochondria incubated in a medium similar to that used to measure flux through GCS, except that radioactive glycine was replaced by unlabelled glycine. Mitochondria were incubated in a final volume of 1.2 ml at a protein concentration of 4 mg/ml. Incubation was carried out for 6 min at 30 °C, at which time 15 μl of $^3\text{H}_2\text{O}$ (20 $\mu\text{Ci/ml}$) and 15 μl of [^{14}C]sucrose (10 $\mu\text{Ci/ml}$) were added. An 0.5 ml portion was immediately transferred to Eppendorf centrifuge tubes (1.5 ml), which contained 100 μl of 15% HClO_4 overlaid by 0.7 ml of a mixture of bromododecane and dodecane (20:1, v/v). Mitochondria were separated from the medium through the oil layer into the acid layer by centrifugation in an Eppendorf centrifuge (10000 g for 1 min.). The acid layer was counted for ^3H and ^{14}C radioactivity by using ScintiVerse E (Fisher Scientific, Toronto, Ontario, Canada) scintillation fluid in a LKB 1214 scintillation counter. Corrections were made for both ^3H overspill into the ^{14}C channel and ^{14}C overspill into the ^3H channel. The mitochondrial matrix space was calculated as the difference between the $^3\text{H}_2\text{O}$ and the [^{14}C]sucrose spaces. The protein sedimented by centrifugation of mitochondria was determined in a parallel series of experiments in which the mitochondrial suspension (0.5 ml) was taken from the same incubation flasks. In this case, Eppendorf tubes used for separating mitochondria contained 1.25 M-KCl in place of HClO_4 . The protein in the pellet was measured by the biuret procedure.

ADP was present in the medium in all the experiments reported. However, the response of mitochondria to all the treatments described in the paper were essentially the same when ADP was omitted from the incubation (results not shown).

RESULTS

It has been suggested that several of the effects of hormones and calcium on mitochondria are mediated by an increase in mitochondrial matrix volume (Halestrap, 1989), and such effects can be manifested by incubation of the mitochondria in hypo-osmotic media. Fig. 1 shows data from experiments where mitochondria were incubated in iso-osmolar and hypo-osmolar media. The flux through GCS was markedly stimulated by hypo-osmolarity. The changes in flux through GCS were accompanied by parallel changes in mitochondrial matrix volume.

Halestrap and others have proposed a mechanism by which hormones such as glucagon, catecholamines and vasopressin regulate hepatic mitochondrial metabolism (see Halestrap, 1989). According to this scheme, these hormones increase cytosolic free Ca^{2+} concentration, which in turn increases mitochondrial matrix volume by stimulating electrogenic entry of potassium into mitochondria. This Ca^{2+} -induced mitochondrial swelling is dependent on phosphate which enters mitochondria as a compensating anion. Data presented in Table 1 show that a sub-micromolar concentration of Ca^{2+} stimulates flux through GCS in isolated mitochondria and that this stimulation by Ca^{2+} is dependent on the presence of potassium phosphate, since its removal from the medium abolished the effect. However, the mitochondrial matrix volume was not affected by Ca^{2+} in either the presence or the absence of phosphate. Potassium phosphate also stimulated the basal flux (i.e. flux in absence of Ca^{2+}), but to a much smaller degree. To examine further the relationship between stimulation of glycine decarboxylation by Ca^{2+} and mitochondrial matrix volume, mitochondria were incubated in hyper- and hypo-osmolar conditions in the presence (0.5 μM) and absence of free Ca^{2+} (Table 2). Hyper-osmolarity decreased the mitochondrial matrix volume by 30 %, and hypo-osmolarity

Fig. 1. Effects of hypo-osmolarity on decarboxylation of glycine and matrix volume in isolated mitochondria

Isolated mitochondria were incubated as described in the Materials and methods section. The osmolarity of the medium was varied by varying the KCl concentration. Data are expressed as means $\pm$ S.E.M. of four separate experiments: * $P < 0.05$, compared with rates under iso-osmotic conditions (by t test). Key: $\circ$, phosphate absent; $\bullet$, phosphate present.

Table 1. Effects of Ca^{2+} and phosphate on flux through GCS and on mitochondrial matrix volume

Mitochondria were incubated as described in the Materials and methods section. Each value is expressed as mean $\pm$ S.E.M. of four separate experiments: * indicates significantly different value ($P < 0.05$, paired t test) compared with values in the absence of Ca^{2+} .

Phosphate...	Flux through GCS (nmol/min per mg of protein)		Mitochondrial matrix volume (μl /mg of protein)	
	0	2.5 mM	0	2.5 mM
0 Ca^{2+}	0.029 $\pm$ 0.002	0.060 $\pm$ 0.010	1.06 $\pm$ 0.06	1.06 $\pm$ 0.10
0.5 μM free Ca^{2+}	0.037 $\pm$ 0.034	0.49 $\pm$ 0.034*	1.04 $\pm$ 0.05	1.10 $\pm$ 0.05

increased matrix volume by 20%. These changes in matrix volume were accompanied by a 40% decrease (hyper-osmolarity) and a 6-fold increase (hypo-osmolarity) in flux through GCS. However, a further stimulation of flux through GCS by 10-fold (hyper-osmolarity) or by 2-fold (hypo-osmolarity) was brought about by 0.5 μM free Ca^{2+} without any further change in matrix volume.

The requirement of phosphate for stimulation of flux through GCS by Ca^{2+} was quite specific, as replacement of phosphate with other permeant anions, such as thiocyanate or acetate, did not permit stimulation of flux through GCS by Ca^{2+} (Table 3).

Experiments were conducted to test whether stimulation of GCS by Ca^{2+} is dependent on the presence of K^+ in the medium (Table 4). In these experiments KCl was replaced by an osmotically equivalent amount of sucrose, and KH_2PO_4 was replaced by Tris phosphate (H_3PO_4 neutralized with Tris base to pH 7.2). Control flasks contained an equivalent amount of Tris base (adjusted to pH 7.2 with HCl). Ca^{2+} stimulated flux through GCS even in the absence of K^+ from the medium as long as phosphate was provided in the medium (Table 4). However, the stimulated flux in these experiments was only about half the rates measured when KCl was present in the medium (Table 1).

Mitochondria isolated from rats injected with glucagon 25 min before killing show elevated rates of flux through GCS (Jois *et al.*, 1989). Studies were carried out to determine whether this stimulated flux was also dependent on phosphate. Table 5 shows data which demonstrate that the mitochondria isolated from rats previously injected with glucagon show elevated rates only when phosphate is present in the medium. Potassium thiocyanate or potassium acetate could not replace potassium phosphate. Mitochondria isolated from sham-injected rats also show higher rates when phosphate is present in the medium compared with rates when phosphate is replaced by potassium acetate or potassium thiocyanate. This effect of phosphate in the sham-injected rats

Table 3. Effect of replacement of phosphate in the medium by either thiocyanate or acetate on the stimulation by Ca^{2+} of flux through GCS

Isolated mitochondria were incubated as described in the text in the presence of 0.5 μM free Ca^{2+} . Each value is presented as mean $\pm$ S.E.M. of four separate experiments: * indicates significantly different value ($P < 0.05$) compared with the flux in the presence of potassium phosphate.

	Flux through GCS (nmol/min per mg of protein)
None	0.13 $\pm$ 0.049*
2.5 mM-potassium phosphate	0.45 $\pm$ 0.062
2.5 mM-potassium thiocyanate	0.16 $\pm$ 0.006*
2.5 mM-potassium acetate	0.17 $\pm$ 0.006*

Table 4. Stimulation of glycine decarboxylation in isolated mitochondria by Ca^{2+} in the absence of K^+

Mitochondria were incubated in a medium in which KCl was iso-osmotically replaced by sucrose. Potassium phosphate was replaced by 2.5 mM- H_3PO_4 neutralized by Tris base where indicated. Control flasks contained an equivalent amount of Tris base neutralized with HCl. When present, the concentration of free Ca^{2+} was 0.5 μM . Data are presented as means $\pm$ S.E.M. of four separate experiments: † $P < 0.05$ compared with rates in absence of phosphate and Ca^{2+} ; * $P < 0.05$ compared with the respective controls without Ca^{2+} .

Ca^{2+}	Tris phosphate	Flux through GCS (nmol/min per mg of protein)
—	—	0.021 $\pm$ 0.006
—	+	0.038 $\pm$ 0.008†
+	—	0.072 $\pm$ 0.006*
+	+	0.21 $\pm$ 0.022*

was relatively minor, however. The mitochondrial matrix volume was not affected by either glucagon treatment or the presence of phosphate, acetate or thiocyanate.

It has been suggested that the primary effect of hormones such as glucagon, catecholamines and vasopressin on mitochondrial metabolism is the stimulation of respiration, which in turn would lead to the other observed effects, such as increased transmembrane pH gradient, membrane potential, pyruvate transport, phosphate transport, ATP/ADP ratio, pyruvate carboxylation, citrulline synthesis, etc. (Yamazaki, 1975; Yamazaki *et al.*, 1980; Halestrap, 1989). The stimulation of gluconeogenesis (from lactate), oxygen consumption and pyruvate carboxylation by glucagon have been shown to be

Table 2. Effect of hyper- and hypo-osmolarity of the medium on the stimulation by Ca^{2+} of flux through GCS

Mitochondria were incubated as described in the Materials and methods section. Data are expressed as means $\pm$ S.E.M. of four separate experiments: † indicates a significant difference from values obtained under iso-osmolar (310 mosM) conditions ($P < 0.05$, paired t test), and * indicates significant difference compared with values in the absence of free Ca^{2+} ($P < 0.05$, paired t test).

Osmolarity of medium (mosM)...	Flux through GCS (nmol/min per mg of protein)			Mitochondrial matrix volume (μl /mg of protein)		
	210	310	410	210	310	410
0 Ca^{2+}	0.45 $\pm$ 0.09†	0.07 $\pm$ 0.01	0.04 $\pm$ 0.02†	1.25 $\pm$ 0.04†	1.06 $\pm$ 0.10	0.74 $\pm$ 0.35†
0.5 μM free Ca^{2+}	1.10 $\pm$ 0.19*	0.82 $\pm$ 0.06*	0.48 $\pm$ 0.25*	1.31 $\pm$ 0.13	1.10 $\pm$ 0.19	0.83 $\pm$ 0.32

Table 5. Effect of phosphate on the stimulation of flux through GCS and on matrix volume in mitochondria isolated from rats previously injected with glucagon

Rats were injected intraperitoneally with glucagon 25 min before being killed; control rats were injected with saline. Mitochondria were incubated as described in the Materials and methods section. Potassium phosphate was replaced, when indicated, by equal concentrations of potassium thiocyanate or of potassium acetate as described in the text. Data are presented as means $\pm$ S.E.M. of three separate experiments, except for experiments involving glucagon injection and measurement of flux through GCS, where data are means $\pm$ S.E.M. of four separate experiments: * $P < 0.05$ compared with control.

	Flux through GCS (nmol/min per mg of protein)		Mitochondrial matrix volume (μ l/mg of protein)	
	Control	Glucagon	Control	Glucagon
None	0.025 $\pm$ 0.002	0.05 $\pm$ 0.004*	1.04 $\pm$ 0.14	1.06 $\pm$ 0.11
KH ₂ PO ₄	0.068 $\pm$ 0.006	0.23 $\pm$ 0.03*	0.96 $\pm$ 0.13	0.96 $\pm$ 0.06
KSCN	0.022 $\pm$ 0.002	0.05 $\pm$ 0.003*	0.91 $\pm$ 0.19*	0.97 $\pm$ 0.03
Potassium acetate	0.032 $\pm$ 0.007	0.07 $\pm$ 0.006*	1.08 $\pm$ 0.35*	1.32 $\pm$ 0.18

Table 6. Effect of inhibition of respiration on glycine decarboxylation and matrix volume in mitochondria isolated from sham- and glucagon-injected rats

Rats were injected intraperitoneally with glucagon (0.1 mg/100 g body wt.) 25 min before being killed. Mitochondria were incubated as described in the Materials and methods section. When present, rotenone was at a concentration of 10 μ M and acetoacetate at 10 mM. Data are presented as means $\pm$ S.E.M. of four separate experiments: * $P < 0.05$, compared with rates in sham-injected rats.

Rotenone	Acetoacetate	Flux through GCS (nmol/min per mg of protein)		Mitochondrial matrix volume (μ l/mg of protein)	
		Control	Glucagon	Control	Glucagon
—	—	0.068 $\pm$ 0.006	0.65 $\pm$ 0.05*	0.96 $\pm$ 0.13	0.96 $\pm$ 0.06
+	—	0.003 $\pm$ 0.002	0.019 $\pm$ 0.002*	1.07 $\pm$ 0.28	1.19 $\pm$ 0.09
+	+	0.074 $\pm$ 0.008	0.54 $\pm$ 0.07*	1.03 $\pm$ 0.18	1.05 $\pm$ 0.11

reversed by a respiratory-chain inhibitor, amytal (Halestrap, 1986; Halestrap & Armston, 1984). We tested the effect of rotenone, an inhibitor of NADH oxidation, on flux through GCS in mitochondria isolated from sham- and glucagon-injected rats (Table 6). Rotenone inhibited flux through GCS, which is to be expected, since by inhibiting NADH oxidation it denies the GCS of NAD⁺. However, when acetoacetate was provided along with rotenone to provide a means of regenerating NAD⁺ (via endogenous β -hydroxybutyrate dehydrogenase) without permitting electron flow through the respiratory chain, the stimulated flux through GCS in mitochondria from rats previously injected with glucagon was evident. These experiments demonstrate that the stimulation of GCS by glucagon is not reversed by inhibition of respiration. It can also be seen that stimulation of flux through GCS by glucagon in these experiments was much higher than that reported in Table 2, owing to the higher dose of glucagon injected in these experiments (100 μ g/100 g body wt., versus 33 μ g/100 g body wt. used in experiments reported in Table 5). Mitochondrial matrix volume was not affected by either rotenone or acetoacetate.

DISCUSSION

A number of studies, especially those by Kikuchi and co-workers, have established hepatic GCS as the major pathway of catabolism of glycine in most vertebrates, including mammals. Three separate mechanisms have been proposed for the regulation of GCS. One proposal suggests inhibition of GCS by branched-chain α -oxo acids (O'Brien, 1978; Kochi *et al.*, 1986), and a second proposal suggests regulation of GCS by changes in the oxidation-reduction state of mitochondrial nicotinamide

nucleotides, a more reduced state inhibiting the flux through GCS (Hampson *et al.*, 1983, 1984). These regulatory mechanisms are unlikely to be physiologically relevant. For example, feeding a high-protein diet leads to stimulation of flux through GCS (Matsuda *et al.*, 1973; Petzke *et al.*, 1986) and is accompanied by increased circulating levels of branched-chain α -oxo acids (Schauder, 1984) and decreased mitochondrial NAD⁺/NADH ratio (Peret *et al.*, 1981; Robinson *et al.*, 1981), both of which would inhibit GCS according to these proposals. A third mechanism, proposed by us, involves hormonal regulation of glycine catabolism and may play an important role in the physiological regulation of GCS (Jois *et al.*, 1989, 1990a,b). The flux through GCS is regulated by hormones and drugs which increase cytosolic cyclic AMP levels as well as by agonists and hormones which increase the cytosolic free Ca²⁺ concentration.

The studies presented here describe the regulation of glycine catabolism in isolated mitochondria. Increased flux through GCS occurred in mitochondria incubated with 0.5 μ M Ca²⁺ concentration, mitochondria isolated from rats previously injected with glucagon and mitochondria incubated under hypo-osmotic conditions. The stimulation of flux through GCS by Ca²⁺ and by injection of glucagon to rats before killing were dependent on the presence of phosphate in the medium. The need for P_i was not simply as a permeant anion, as other permeant anions such as acetate and thiocyanate could not replace P_i. The requirement for phosphate was not due to a requirement for oxidative phosphorylation, as the stimulated flux could be demonstrated when respiration was inhibited by rotenone. Also, the requirement for phosphate was evident when ADP was omitted from the medium.

These studies also demonstrate that it is possible to separate

stimulation of respiration and stimulation of flux through GCS. Additional experiments are needed to examine which effects of hormones are dependent on concomitant stimulation of respiration. The present studies also show that the stimulation of flux through GCS by Ca^{2+} as well as glucagon can occur in a medium devoid of K^+ .

Halestrap and colleagues have proposed a scheme for hormonal action on mitochondrial metabolism where an increase in the concentration of cytosolic free Ca^{2+} increases K^+ permeability of mitochondria, leading to a volume increase and consequent metabolic effects (see Halestrap, 1989). However, this scheme does not appear to be involved in the regulation of GCS. Our results, as well as those of Halestrap *et al.* (1990), show that the stimulation of flux through GCS by hypo-osmolar conditions is accompanied by parallel changes in mitochondria matrix volume. However, the changes in flux through GCS in isolated mitochondria brought about by $0.5 \mu\text{M}$ free Ca^{2+} , as well as the stimulation of flux through GCS in mitochondria isolated after injection of glucagon *in vivo*, are independent of change in mitochondrial matrix volume. In fact, mitochondria incubated under hyper-osmotic conditions in the presence of $0.5 \mu\text{M}$ free Ca^{2+} exhibited decreased matrix volume but markedly elevated flux through GCS as compared with mitochondria incubated under iso-osmotic conditions in the absence of free Ca^{2+} (Table 2). In addition, hypo-osmolarity did not abolish the stimulation of flux through GCS by $0.5 \mu\text{M}$ free Ca^{2+} . Our results, showing no change in the matrix volume of mitochondria isolated from glucagon-treated rats, is in agreement with Hamman & Haynes (1983) and Wingrove *et al.* (1984), but in disagreement with Armston *et al.* (1982), who showed a 13% increase. Although Halestrap and co-workers conclude that Ca^{2+} increases mitochondrial matrix volume, based on light-scattering measurements in mitochondria and isolated hepatocytes (see Halestrap, 1989), direct measurement of mitochondrial matrix volume by Wingrove *et al.* (1984) showed no effect of $1.5 \mu\text{M}$ free Ca^{2+} . It must be pointed out, however, that the discrepancy could be due to difficulties in measuring small changes in matrix volume. For example, it can be calculated from the standard error of matrix volume in unstimulated mitochondria (Table 1) that a change in matrix volume of less than $0.2 \mu\text{l}/\text{mg}$ of protein (a 20% change) could not have been detected with a confidence limit of 95%. However, our experiments in which matrix volume was measured in mitochondria exposed to hyper- and hypo-osmolar medium in the presence of Ca^{2+} strongly suggest that the

effect of Ca^{2+} on glycine oxidation is largely independent of changes in matrix volume. Further experiments are needed to elucidate the mechanism by which hormones and Ca^{2+} stimulate flux through GCS.

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REFERENCES

- Armston, A. E., Halestrap, A. P. & Scott, R. D. (1982) *Biochim. Biophys. Acta* **681**, 429–439
- Denton, R. M., Richards, D. A. & Chin, J. G. (1978) *Biochem. J.* **176**, 899–906
- Gornall, A. G., Bardwill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766
- Halestrap, A. P. (1986) in *Hormonal Control of Gluconeogenesis* (Kraus-Friedman, N., ed.), vol. 3, pp. 31–48, CRC Press, Boca Raton, FL
- Halestrap, A. P. (1989) *Biochim. Biophys. Acta* **973**, 365–382
- Halestrap, A. P. & Armston, A. E. (1984) *Biochem. J.* **223**, 677–685
- Halestrap, A. P., Davidson, A. M. & Potter, W. D. (1990) *Biochim. Biophys. Acta* **1018**, 278–281
- Hamman, H. C. & Haynes, R. C., Jr. (1983) *Biochim. Biophys. Acta* **724**, 241–250
- Hampson, R. K., Barron, L. L. & Olson, M. S. (1983) *J. Biol. Chem.* **258**, 2993–2999
- Hampson, R. K., Taylor, M. K. & Olson, M. S. (1984) *J. Biol. Chem.* **259**, 1180–1185
- Jois, M., Hall, B., Fewer, K. & Brosnan, J. T. (1989) *J. Biol. Chem.* **264**, 3347–3351
- Jois, M., Hall, M. & Brosnan, J. T. (1990a) *J. Biol. Chem.* **265**, 1246–1248
- Jois, M., Hall, B., Collett, V. M. & Brosnan, J. T. (1990b) *Biochem. Cell Biol.* **68**, 543–546
- Kikuchi, G. (1973) *Mol. Cell. Biochem.* **1**, 169–187
- Kochi, H., Seino, H. & Ono, K. (1986) *Arch. Biochem. Biophys.* **249**, 263–272
- Matsuda, Y., Kuroda, Y., Kobayashi, K. & Katuama, N. (1973) *J. Biochem. (Tokyo)* **73**, 291–298
- O'Brien, W. E. (1978) *Arch. Biochem. Biophys.* **189**, 291–297
- Peret, J., Foustock, S., Chanez, M., Bois-Joyeux, B. & Assan, R. (1981) *J. Nutr.* **111**, 1175–1184
- Petzke, K. J., Albrecht, V. & Pzbilski, H. (1986) *J. Nutr.* **116**, 742–750
- Robinson, J. L., Foustock, S., Chanez, M., Bois-Joyeux, B. & Peret, J. (1981) *J. Nutr.* **111**, 1711–1720
- Schauder, P. (1984) in *Branched Chain Amino Acids and Keto Acids in Health and Disease* (Adibi, S. A., Fekl, W., Langenbeck, U. & Schauder, P., eds.), pp. 228–241, Karger, Basel
- Wingrove, D. E., Amatruda, J. M. & Gunter, T. E. (1984) *J. Biol. Chem.* **259**, 9390–9394
- Yamazaki, R. K. (1975) *J. Biol. Chem.* **250**, 7924–7930
- Yamazaki, R. K., Mickey, D. L. & Story, M. (1980) *Biochim. Biophys. Acta* **292**, 1–12

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