Biochem. J. (1985) 232, 593–597 (Printed in Great Britain)

Activation of rat liver branched-chain 2-oxo acid dehydrogenase *in vivo* by glucagon and adrenaline

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The activity of liver branched-chain 2-oxo acid dehydrogenase complex was measured in rats fed on low-protein diets and given adrenaline, glucagon, insulin or dibutyryl cyclic AMP *in vivo*. Administration of glucagon or adrenaline (200 μg/100 g body wt.) resulted in a 4-fold increase in the percentage of active complex. As with glucagon and adrenaline, treatment of rats with cyclic AMP (5 mg/100 g body wt.) resulted in marked activation of branched-chain 2-oxo acid dehydrogenase. Insulin administration (1 unit/100 g body wt.) also resulted in activation of enzyme; however, these effects were less than those observed with glucagon and adrenaline. In contrast with the results obtained with low-protein-fed rats, administration of adrenaline (200 μg/100 g body wt.) to rats fed with an adequate amount of protein resulted in only a modest (14%) increase in the activity of the complex. The extent to which these hormones activate branched-chain 2-oxo acid dehydrogenase appears to be correlated with their ability to stimulate amino acid uptake into liver.

INTRODUCTION

Most reports on hormonal control of branched-chain amino acid catabolism have dealt with measurements of flux of ^{14}C-labelled branched-chain amino or oxo acids in isolated intact tissues (Harper *et al.*, 1984). Buse *et al.* (1973) studied the effects of glucagon and adrenaline on branched-chain amino acid oxidation in isolated hemidiaphragms and perfused hearts from rats. Depending on the tissue studied, the nutritional state of the rats and the presence or absence of glucose in the media, glucagon and adrenaline increased or had no effect on catabolism of 1-^{14}C-labelled branched-chain amino acids. More recently, α-adrenergic inhibition of oxidation of 1-^{14}C-labelled branched-chain 2-oxo acids in perfused liver (Buxton *et al.*, 1982) and oxidation of 1-^{14}C-labelled branched-chain L-amino acids in isolated diaphragm (Palmer *et al.*, 1983) have been reported. In all of these studies, the effects of hormones on branched-chain 2-oxo acid dehydrogenase activity cannot be separated from effects on preceding reactions such as plasma- and mitochondrial-membrane substrate transport, branched-chain amino acid aminotransferase activity, and protein synthesis and degradation. Apart from the report of insulin modulation of adipose-tissue enzyme (Frick & Goodman, 1980), there have been few studies where the activation state of branched-chain 2-oxo acid dehydrogenase has been measured after hormone treatment. In contrast, numerous studies have shown that the activity of the structurally similar pyruvate dehydrogenase complex is modulated by specific hormones (Saltiel *et al.*, 1981; Wieland, 1983; Oviasu & Whitton, 1984). In the present paper, effects of short-term administration of adrenaline, glucagon and insulin on the activity and percentage of active hepatic branched-chain 2-oxo acid dehydrogenase complex in rats are reported. Previous studies have shown that hepatic branched-chain 2-oxo acid dehydrogenase is essentially fully activated (i.e. dephosphorylated) in rats fed on standard laboratory chow, but only partially active in rats fed on low-protein diets (Gillim *et al.*, 1983; Wagenmakers *et al.*, 1984; Patston *et al.*, 1984; Block *et al.*, 1985). Therefore the effects of hormones on liver branched-chain 2-oxo acid dehydrogenase were investigated in rats fed on low-protein diets, on the assumption that changes in the activation state would be more easily detectable.

MATERIALS AND METHODS

Materials

Crystalline bovine insulin and glucagon were kindly provided by Dr. W. W. Bromer (Eli Lilly and Co., Indianapolis, IN, U.S.A.); Tos-Lys-CH_2Cl (tosyl-lysyl-chloromethane hydrochloride) and pelleted 9%-casein diet were from United States Biochemical Corp. (Cleveland, OH, U.S.A.); (−)-adrenaline (+)-bitartrate, N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate (dibutyryl cyclic AMP; sodium salt), catalase, L-amino acid oxidase (type IV), β-NAD^+ (grade III), CoA (lithium salt, grade III-L), thiamin pyrophosphate, DL-dithiothreitol, leupeptin, polyoxyethylene ether W-1 and 3-methyl-2-oxobutanoate (sodium salt) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); L-[1-^{14}C]valine (47.9 mCi/mmol) was from New England Nuclear (Boston, MA, U.S.A.); DL-2-chloro-4-methylpentanoate was given by Dr. R. J. Strohscheim and Dr R. Simpson (Sandoz, East Hanover, NJ, U.S.A.), and glucose oxidase–peroxidase kit was from Boehringer–Mannheim (Indianapolis, IN, U.S.A.). 3-Methyl-2-oxo-[1-^{14}C]butanoate was prepared from L-[1-^{14}C]valine by using L-amino acid oxidase and catalase as described by Rudiger *et al.* (1972). All other chemicals were at least reagent grade.

Care and treatment of animals

The type and care of animals was as described by May *et al.* (1980). Male Wistar rats (Charles River Breeding

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Laboratories, Wilmington, MA, U.S.A.), weighing approx 200 g, were fed on a low-protein diet (United States Biochemical Corp.) which was identical with that described by Wohlhueter & Harper (1970), except that it was pelleted and contained AIN vitamin mixture 76 in place of the formula described by Rogers & Harper (1965). In other experiments, rats were fed on a standard laboratory chow containing 24.5% (w/w) protein (Wayne Laboratories, Chicago, IL, U.S.A.). Diets were fed *ad libitum* for 14–18 days before enzyme activities were measured. Solutions of insulin, glucagon, adrenaline and dibutyryl cyclic AMP were prepared in 0.9% NaCl on the day of the study. To ensure that rats were in the postabsorptive state, food was withdrawn 15 h before administration of solutions. All solutions were administered intraperitoneally. Rats were killed by decapitation 30 min after injections for assay of liver branched-chain 2-oxo acid dehydrogenase activity.

Assay of branched-chain 2-oxo acid dehydrogenase

Liver branched-chain 2-oxo acid dehydrogenase activity was measured with an assay system containing detergent, to solubilize fully mitochondrial membranes, and KF (phosphatase inhibitor) and 2-chloro-4-methylpentanoate (kinase inhibitor), to retard interconversion of the active–inactive forms of the complex. Two separate 10% (w/v) homogenates were prepared from each rat liver. The basal (non-activated) homogenate contained 220 mM-D-mannitol, 70 mM-sucrose, 4 mM-Hepes, 0.2 mM-EGTA, and 100 mM-KF plus 0.5 mM-2-chloro-4-methylpentanoate. The activity of the complex assayed in this buffer was used to estimate the activity state present *in vivo*. The second homogenate was prepared in the same buffer, with the inhibitors of the phosphatase and kinase omitted. The activity of the complex assayed in this buffer after preincubation was used to estimate total (fully activated) branched-chain 2-oxo acid dehydrogenase activity. Homogenates for measuring basal complex activity were always prepared first, to minimize interconversion of enzyme. Homogenates were kept on ice in a room maintained at 4 °C until time of assay. The final assay medium (pH 7.4 at 37 °C) contained in 0.4 ml: 3-methyl-2-oxo[1-^{14}C]butanoate (2 mM, 140 d.p.m./nmol); NAD^+ (1.5 mM); CoA (1 mM); thiamin pyrophosphate (0.4 mM); dithiothreitol (5 mM); polyoxyethylene ether W-1 detergent (0.5%); Hepes (50 mM); EGTA (2 mM); Na_2CO_3 (5 mM); leupeptin (20 μg/ml); and Tos-Lys-CH_2Cl (1 mM). 3-Methyl-2-oxobutanoate (ketovaline) was chosen as the substrate for measuring branched-chain 2-oxo acid dehydrogenase in whole homogenates, as Sabourin & Bieber (1981) and others have demonstrated negligible extramitochondrial oxidation of this substance in liver. The assays for basal enzyme activity contained, in addition to these components, a final concentration of 22.5 mM-KF and 0.1 mM-2-chloro-4-methylpentanoate (from the homogenate). KF and 2-chloro-4-methylpentanoate were therefore added to the total enzyme assay media so that basal and total enzyme assays contained the same components at identical concentrations. Addition of 0.1 mM-2-chloro-4-methylpentanoate to the assay tube had no measurable effect on the oxidation of 3-methyl-2-oxobutanoate. Preliminary studies demonstrated that inclusion of leupeptin (50 μM), Tos-Lys-CH_2Cl (1 mM) (serine-proteinase inhibitor) and/or calf serum (1%, v/v) had no significant effect on the activity of liver branched-chain 2-oxo acid dehydrogenase in our assay system (results not shown). A similar lack of effect of proteinase inhibitors on branched-chain 2-oxo acid dehydrogenase was shown previously by May *et al.* (1980) in intact and freeze-thawed liver mitochondria. As a precautionary measure, leupeptin and Tos-Lys-CH_2Cl were included in the final assay system. Reactions were begun by adding 0.1 ml of homogenate and terminated after 2 min with 0.2 ml of 25% (w/v) trichloroacetic acid. For determinations of total enzyme activity, samples of the homogenate not containing phosphatase and kinase inhibitors were incubated in 2 mM-$MgCl_2$ at 37 °C for 30 min before assay. Preliminary studies demonstrated that 30 min of preincubation gave maximal branched-chain 2-oxo acid dehydrogenase activities in low-protein-fed and stock-diet-fed rats.

In some preliminary studies, branched-chain 2-oxo acid dehydrogenase activities were assayed by the method of Wohlhueter & Harper (1970) with modifications as described by Block *et al.* (1985). Both assay systems consistently released over 1000 c.p.m. above background, were run in triplicate and were linear with respect to time and enzyme amount.

RESULTS

Effects of glucagon, adrenaline and insulin on branched-chain 2-oxo acid dehydrogenase activity in rats fed on low-protein diets

The effects of hormones on the activity of liver branched-chain 2-oxo acid dehydrogenase are given in Table 1. Administration of glucagon (200 μg/100 g body wt.) to rats fed on low-protein diets resulted in a greater than 4-fold increase in basal (non-preincubated) branched-chain 2-oxo acid dehydrogenase activity. Accompanying this stimulation was an increase in the percentage of active complex from 17% to 64% (Table 1). Treatment of low-protein-fed rats with adrenaline (200 μg/100 g body wt.) also resulted in marked increases in basal activity and percentage of active branched-chain 2-oxo acid dehydrogenase (Table 1). Preliminary experiments were conducted to investigate the effects of administering different concentrations of glucagon or adrenaline on the enzyme from low-protein-fed rats (Block, 1984). These studies employed the Ca^{2+}-swollen mitochondrial assay system originally described by Wohlhueter & Harper (1970), with subsequent modifications by Block *et al.* (1985). Data from these studies were qualitatively in accord with the results given in Table 1, in that administration of glucagon and adrenaline (200 μg/100 g body wt.) resulted in marked activation of liver branched-chain 2-oxo acid dehydrogenase from low-protein-fed rats. A significant dose-dependent increase ($r = 0.696$, $P < 0.01$) in basal complex activity was observed when the dosage of adrenaline was varied from 25 to 100 μg/100 g body wt. (results not shown). In contrast, administration of glucagon at a dosage as low as 10 μg/100 g body wt. resulted in near-full activation of the enzyme from low-protein-fed rats. Treatment of rats with dibutyryl cyclic AMP (5 mg/100 g body wt.) also resulted in near-full activation of the complex (results not shown). The results of these preliminary experiments indicate that glucagon is approx. 100 times more potent than adrenaline in its ability to activate liver branched-chain 2-oxo acid dehydrogenase from low-protein-fed rats, and that these effects may be mediated via cyclic AMP.

Table 1. Liver branched-chain 2-oxo acid dehydrogenase activity in rats fed on a low-protein diet and given glucagon, adrenaline or insulin *in vivo*

Rats (160–180 g) were fed on a low-protein diet *ad libitum* for 14–18 days. Glucagon (200 μg/100 g body wt.), adrenaline (200 μg/100 g body wt.) or insulin (1 unit/100 g body wt.) was administered intraperitoneally to rats (254–320 g) previously kept without food for 15 h. Rats receiving only saline (0.9% NaCl) served as the control. Rats were killed 30 min after injections, and basal (no preincubation) and total (+ preincubation) branched-chain 2-oxo acid dehydrogenase activities were measured as described in the Materials and methods section. Enzyme activities are expressed as nmol of 3-methyl-2-oxo[1-^{14}C]butanoate oxidized/min per g of liver. The '% active complex' was calculated as: 100 × (activity without preincubation/activity + preincubation). Values represent means ± S.E.M. for four (saline, adrenaline and insulin treatments) or five (glucagon treatment) rats. $*P < 0.05$ and $**P < 0.01$ for significance of differences from values obtained with saline injections.

Treatment	Branched-chain 2-oxo acid dehydrogenase activity		
	No preincubation	+ Preincubation	% active complex
Saline	285 ± 33	1669 ± 87	17 ± 2
Glucagon	1210 ± 36**	1912 ± 111	64 ± 4**
Adrenaline	919 ± 67**	1347 ± 81*	69 ± 3**
Insulin	842 ± 94**	1583 ± 65	53 ± 5**

Table 2. Liver branched-chain 2-oxo acid dehydrogenase activity in rats fed with adequate amounts of protein and given adrenaline *in vivo*

Rats (181–204 g) were fed on a standard laboratory chow diet (24.5% protein) up until the time of the experiment. Adrenaline (200 μg/100 g body wt.) or saline was administered intraperitoneally to rats previously denied food for 15 h. Rats were killed 30 min after injection for assay of enzyme activity. Assay conditions were identical with those described in Table 1. Values represent means ± S.E.M. for six (saline treatment) or four (adrenaline treatment) rats. $*P < 0.05$ and $**P < 0.01$ for significance of differences from values obtained with saline injections.

Treatment	Branched-chain 2-oxo acid dehydrogenase activity		
	No preincubation	+ Preincubation	% active complex
Saline	1509 ± 46	2177 ± 67	69 ± 1
Adrenaline	1724 ± 73*	2245 ± 121	77 ± 2**

Treatment of rats with insulin (1 unit/100 g body wt.) also resulted in activation of the complex (Table 1). However, the increases in basal activity and percentage of active enzyme tended to be lower in insulin-treated rats than in rats given glucagon and adrenaline. Plasma glucose concentrations of saline-treated controls and insulin-treated rats were 145 ± 4 and 51 ± 4 mg/dl respectively (means ± S.E.M. for $n = 4$ rats/group). Hence changes in branched-chain 2-oxo acid dehydrogenase after insulin treatment may be due to the elevated concentrations of glucagon which normally occur during hypoglycaemic episodes.

Effects of adrenaline on branched-chain 2-oxo acid dehydrogenase activity in rats fed with adequate amounts of protein

Experiments were also conducted on stock-diet-fed rats (Table 2). Hepatic branched-chain 2-oxo acid dehydrogenase was approx. 70% active in rats fed on a standard laboratory chow containing approx. 25% protein. Decreasing the protein content of the diet from 25% to 9% resulted in a significant ($P < 0.01$), although modest (23%), decrease in total branched-chain 2-oxo acid dehydrogenase activity (cf. Table 1 and 2). A significant ($P < 0.05$), but modest (14%), increase in basal enzyme activity was observed after adrenaline was administered to rats fed on an adequate-protein diet (Table 2). Hence increasing the protein content of the diet from 9% to 25% decreased the adrenaline-mediated stimulation of basal branched-chain 2-oxo acid dehydrogenase activity by approx. 95% ($P < 0.01$).

DISCUSSION

Administration of glucagon or adrenaline to low-protein-fed rats resulted in marked increases in the percentage of active branched-chain 2-oxo acid dehydrogenase (Table 1). Also, adrenaline administration resulted in a slight but significant ($P < 0.05$) increase in basal enzyme activity in livers from stock-diet-fed rats (Table 2). These results are in apparent contradiction to those of Buxton *et al.* (1982), who reported inhibition of 4-methyl-2-oxo[1-^{14}C]pentanoate flux through branched-chain 2-oxo acid dehydrogenase in perfused livers after inclusion of adrenaline or other α-adrenergic agents in the perfusate. A similar α-adrenergic inhibition of flux of 1-^{14}C-labelled branched-chain amino acids through branched-chain 2-oxo acid dehydrogenase has been reported in isolated diaphragm (Palmer *et al.*, 1983). The observations by Buxton *et al.* (1982) along with the results of the present study demonstrate the disparity that may exist between flux and enzyme-activity measure-

ments. Similar discrepancies have been observed between [1-^{14}C]pyruvate flux through, and the activity state of, pyruvate dehydrogenase in perfused liver (Sies *et al.*, 1983). In the latter study, perfusion of rat liver with vasopressin resulted in a decreased flux of [1-^{14}C]pyruvate but an increased percentage of active pyruvate dehydrogenase complex. Interestingly, the depression in pyruvate flux was very transient, and was followed by a significant increase in pyruvate flux (Sies *et al.*, 1983). Whether such a time course of events exists for branched-chain 2-oxo acid flux in perfused liver is unknown.

The decrease in total branched-chain 2-oxo acid dehydrogenase activity observed in the present study when rats were switched from an adequate to a low-protein diet (Tables 1 and 2) is qualitatively in accord with previous reports. In the studies by Gillim *et al.* (1983) and Patston *et al.* (1984), a 50–70% decrease in total complex activity was observed when rats were switched from an adequate to a low-protein diet. This is thought to represent a decrease in the total amount of enzyme protein. In the present study, and in previous experiments where the effects of individual amino acids on the activity of branched-chain 2-oxo acid dehydrogenase were measured (Block *et al.*, 1985), consumption of low-protein meals resulted in significant but modest decreases in total complex activity. Our values for fully activated complex from adequate- and low-protein-fed rats were approx. 2200 and 1700 nmol/min per g of liver respectively (Tables 1 and 2) These values for total complex activities from adequate- and low-protein-fed rats are 3 and 9 times respectively the values reported by Gillim *et al.* (1983). The reasons for the discrepancies between our studies and those of Gillim *et al.* (1983) and Patston *et al.* (1984) are unknown. However, as whole homogenates (rather than tissue extracts and mitochondria) were used in our studies, there may be differences in enzyme recovery. Also, rats in the present study were fed on a low-protein diet supplemented with methionine, the most limiting amino acid in casein. Hence rats fed on this diet grew better than did rats fed on a 9%-casein diet alone. Proper quantification of the amount of branched-chain 2-oxo acid dehydrogenase in tissues after hormonal and dietary manipulations is dependent on the development of antibodies that can distinguish between the E1 subunits of the pyruvate dehydrogenase and branched-chain 2-oxo acid dehydrogenase complexes.

Administration of glucagon and adrenaline has been shown to result in hepatic intracellular Ca^{2+} redistribution (Sies *et al.*, 1983; Wingrove *et al.*, 1984). Oviasu & Whitton (1984) reported that pyruvate dehydrogenase in isolated hepatocytes was dephosphorylated on incubation with glucagon, phenylephrine (α-agonist) or vasopressin. Incubation with insulin was without effect. The activation of pyruvate dehydrogenase appeared to be dependent on extracellular Ca^{2+}. More recently, Staddon & McGivan (1985) reported similar findings with oxoglutarate dehydrogenase in isolated hepatocytes incubated with vasopressin. Activation of pyruvate dehydrogenase by these hormones has been attributed to the marked stimulation of pyruvate dehydrogenase phosphatase by intracellular Ca^{2+}. However, a direct effect of intracellular Ca^{2+} redistribution on branched-chain 2-oxo acid dehydrogenase is in question, as Damuni *et al.* (1984) reported that branched-chain 2-oxo acid dehydrogenase phosphatase is non-responsive to Ca^{2+}. Also administration of vasopressin (10 munits/100 g body wt.) *in vivo* failed to alter the activity of liver branched-chain 2-oxo acid dehydrogenase in rats fed on low-protein diets (K. P. Block & M. G. Buse, unpublished work). Nonetheless, a role for Ca^{2+} in the regulation of liver branched-chain 2-oxo acid dehydrogenase has been proposed by numerous investigators (Aftring *et al.*, 1982; Hauschildt *et al.*, 1982; Patel & Olson, 1982; Paul & Adibi, 1983).

Phosphorylation of branched-chain 2-oxo acid dehydrogenase is not cyclic-AMP-mediated (Randle *et al.*, 1984). However, the activation state of branched-chain 2-oxo acid dehydrogenase can be modified by dietary protein (Gillim *et al.*, 1983; Patston *et al.*, 1984) and individual amino acids (Block *et al.*, 1985). Stimulation of amino acid transport into livers of rats after administration of certain hormones *in vivo* has been well documented (Tews *et al.*, 1970; Guidotti *et al.*, 1978). In these studies, glucagon was the most potent stimulator of amino acid transport into liver, followed by adrenaline, cortisol and insulin. Hence the ability of the hormones in the present study to activate branched-chain 2-oxo acid dehydrogenase may be correlated with their stimulatory effects on liver amino acid transport. Alternatively, the activation of the complex observed after high-protein feeding may be mediated by certain hormones. Marked increases in portal-vein blood glucagon concentrations are observed in rats after high-protein feeding (Eisenstein *et al.*, 1979). Starvation, like high-protein feeding, is associated with increases in blood glucagon concentrations (Pozefsky *et al.*, 1976) and liver branched-chain 2-oxo acid dehydrogenase activities (Paul & Adibi, 1982). It is tempting to speculate that the activation of hepatic branched-chain 2-oxo acid dehydrogenase by glucagon serves (*a*) to clear excesses of branched-chain amino acids and 2-oxo acids during high-protein feeding and (*b*) to supply the organism with gluconeogenic and ketogenic substrates during periods of starvation. However, whether or not the relationship between blood glucagon concentration and liver branched-chain 2-oxo acid dehydrogenase activity is causal is unknown at present. Harris *et al.* (1985) have proposed that cyclic AMP may activate the complex in hepatocytes of low-protein-fed rats indirectly by lowering the concentrations of lactate and pyruvate. Also, Randle and associates (Patston *et al.*, 1984; Espinal *et al.*, 1985) have described a protein activator (presumably the free E1 subunit of the complex) which activates branched-chain 2-oxo acid dehydrogenase without dephosphorylation. To what extent hormones alter the activity of this protein is unknown. Clarification of the mechanisms underlying these processes will aid in the understanding of the physiological importance of hormone-mediated increases in branched-chain 2-oxo acid dehydrogenase activity.

This work was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin–Madison, a Postdoctoral Fellowship from the Graduate School of the Medical University of South Carolina (to K.P.B.), and by grants AM10748 and P30 AM26659 (to A.E.H) and grant AM 02001 (to M.G.B.) from the U.S. Public Health Service, National Institutes of Health.

REFERENCES

Aftring, R. P., May, M. E., Manos, P. N & Buse, M. G. (1982) J. Biol. Chem. **257**, 6156–6163

Block, K. P. (1984) Ph.D. Thesis, University of Wisconsin–Madison
Block, K. P., Soemitro, S, Heywood, B. W. & Harper, A. E. (1985) J. Nutr. **115**, in the press
Buse, M. G., Biggers, J. F., Drier, C. & Buse, J. F. (1973) J. Biol. Chem. **248**, 697–706
Buxton, D., Barron, L. L. & Olson, M. S. (1982) J. Biol. Chem. **257**, 14318–14323
Damuni, Z, Merryfield, M. L., Humphreys, J. S. & Reed, L. J. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 4335–4338
Eisenstein, A. B., Strack, I., Gallo-Torres, H., Georgiadis, A. & Miller, O. N. (1979) Am. J. Physiol. **236**, E20–E27
Espinal, J., Patston, P. A., Fatania, H. R., Lau, K. S. & Randle, P. J. (1985) Biochem. J. **225**, 509–516
Frick, G. P. & Goodman, H. M. (1980) J. Biol. Chem. **255**, 6186–6192
Gillim, S. E., Paxton, R., Cook, G. A. & Harris, R. A. (1983) Biochem. Biophys. Res. Commun. **111**, 74–81
Guidotti, G. G, Borghetti, A. F. & Gazzola, G. C. (1978) Biochim. Biophys. Acta **515**, 329–366
Harper, A. E., Miller, R. H. & Block, K. P. (1984) Annu. Rev. Nutr. **4**, 409–454
Harris, R. A., Paxton, R. & Jenkins, P. (1985) Fed. Proc. Fed. Am. Soc. Exp. Biol. **44**, 2463–2468
Hauschildt, S, Neuman, B. & Brand, K. (1982) FEBS Lett. **143**, 77–80
May, M. E., Mancusi, V. J., Aftring, R. P. & Buse, M. G. (1980) Am. J. Physiol. **239**, E215–E222
Oviasu, O. A. & Whitton, P. D. (1984) Biochem. J. **224**, 181–186
Palmer, T. N., Caldecourt, M. A. & Sugden, M. C. (1983) Biochem. J. **216**, 63–70
Patel, T. B. & Olson, M. S. (1982) Biochemistry **21**, 4259–4265
Patston, P. A., Espinal, J. & Randle, P. J. (1984) Biochem. J. **222**, 711–719
Paul, H. S. & Adibi, S. A. (1982) J. Biol. Chem. **257**, 4875–4881
Paul, H. S. & Adibi, S. A. (1983) J. Biol. Chem. **258**, 11471–11475
Pozefsky, T., Tancredi, R. G., Moxley, R. T., Dupre, J. & Tobin, J. D. (1976) J. Clin. Invest. **57**, 444–449
Randle, P. J., Fatania, H. R. & Lau, K. S. (1984) Mol. Aspects Cell. Regul. **3**, 1–26
Rogers, Q. R. & Harper, A. E. (1965) J. Nutr. **87**, 267–273
Rudiger, H. W., Langenbeck, U. & Goedde, H. W. (1972) Biochem. J. **126**, 445–446
Sabourin, P. J. & Bieber, L. L. (1981) Arch. Biochem. Biophys. **206**, 132–144
Saltiel, A., Jacobs, S., Siegel, M. & Cuatrecasas, P. (1981) Biochem. Biophys. Res. Comun. **102**, 1041–1047
Sies, H., Graf, P. & Crane, D. (1983) Biochem. J. **212**, 271–278
Staddon, J. M. & McGivan, J. D. (1985) Biochem. J. **225**, 327–333
Tews, J. K., Woodcock, N. A. & Harper, A. E. (1970) J. Biol. Chem. **245**, 3026–3032
Wagenmakers, A. J. M., Schepens, J. T. G., Veldhuizen, J. A. M. & Veerkamp, J. H. (1984) Biochem. J. **220**, 273–281
Wieland, O. H. (1983) Rev. Physiol. Biochem. Pharmacol. **96**, 123–170
Wingrove, D. E., Amatruda, J. M. & Gunter, T. E. (1984) J. Biol. Chem. **259**, 9390–9394
Wohlhueter, R. M. & Harper, A. E. (1970) J. Biol. Chem. **245**, 2391–2401

Received 14 March 1985/29 July 1985; accepted 9 August 1985