Regulatory Effects of Fatty Acyl-Coenzyme A Derivatives on Phosphate-Activated Pig Brain and Kidney Glutaminase *in vitro*

By ELLING KVAMME and INGEBORG AASLAND TORGNER

Neurochemical Laboratory, The Oslo University Psychiatric Clinic, Vinderen, Oslo 3, Norway

(Received 23 December 1974)

1. Fatty *n*-acyl-CoA derivatives in the concentration range $5\mu\text{M}$ – 0.1mM and with 5–18 fatty acyl carbons have dual effects on phosphate-activated glutaminase from pig brain and kidney. Generally, fatty acyl-CoA derivatives in low concentrations activate the enzyme, but inhibit at higher concentrations; phosphate and citrate potentiate the activation, displaying positive co-operativity, and protect against inactivation. The fatty acyl-CoA derivatives affect glutaminase similarly to Bromothymol Blue, but differently from acetyl-CoA, which activates the enzyme only at very low phosphate or citrate concentrations. 2. Saturated fatty acyl-CoA derivatives, with 5–10 fatty acyl carbons, only activate the enzyme in the concentration range 0 – 0.1mM . When the fatty acyl chain is elongated, the fatty acyl-CoA derivatives gradually become more powerful inhibitors of glutaminase at the expense of their activating capacity. In particular, palmitoyl-CoA and stearoyl-CoA are strong inhibitors at concentrations ($10\mu\text{M}$) at which the corresponding free fatty acids and fatty acyl-carnitine derivatives have no effect. 3. The unsaturated fatty acyl-CoA derivatives, oleoyl-CoA and linoleoyl-CoA, behave as potent activators in the lower part of the concentration range tested (0 – 0.05mM), and as inhibitors in the upper part of this range (0.02 – 0.10mM). Oleic acid and linoleic acid have similar properties, but their activating capacity is less pronounced. 4. Phosphate both prevented and reversed the inhibition, but no restoration of activity was possible once the enzyme became inactivated. 5. By changing the pH from 7.0 to 8.0 the activating capacity of the fatty acyl-CoA derivatives is increased, as is their concentration range for activation. 6. The fatty acyl-CoA derivatives are somewhat more potent activators for brain glutaminase, but otherwise they affect the two enzymes similarly.

Pig kidney and brain phosphate-activated glutaminase (EC 3.5.1.2; L-glutamine amidohydrolase) purified to apparent homogeneity (Kvamme *et al.*, 1970; Svenneby *et al.*, 1973) is activated by a range of different compounds (Svenneby *et al.*, 1970; Svenneby, 1970, 1972; Weil-Malherbe & Beall, 1970; Tveit *et al.*, 1970; Weil-Malherbe, 1972). Among these, the dye Bromothymol Blue is particularly interesting because it behaves as an activator at concentrations below $50\mu\text{M}$, and as an inhibitor when the concentration is increased. Moreover, phosphate potentiates the activation by the dye, and protects against inhibition. It has been shown that Bromothymol Blue probably acts as an activator by increasing the affinity of the enzyme for anionic activators (Kvamme *et al.*, 1965; Kvamme & Torgner, 1974*a,b*). In a search for natural compounds showing the Bromothymol Blue type of action, it has recently been demonstrated that palmitoyl-CoA, as well as stearoyl-CoA, affects glutaminase in a manner similar to that of Bromothymol Blue. However, at low phosphate concentrations ($<7\text{mM}$) and pH 7.0 , these compounds act predominantly as inhibitors (Kvamme & Torgner,

1974*b*). The effect of palmitoyl-CoA and stearoyl-CoA on glutaminase is completely different from that of acetyl-CoA (Kvamme & Torgner, 1974*a*), because the activation by acetyl-CoA is maximally effective in the absence of phosphate.

In the present paper, the effects of acyl-CoA derivatives with 5–18 fatty acyl carbons are described. In principle they all show the Bromothymol Blue type of action on glutaminase. However, the relationship between their activating and inhibiting properties varies with their fatty acyl residue. Generally, acyl-CoA derivatives are more effective inhibitors when the fatty acyl chain is elongated and more effective activators on unsaturation of the fatty acyl group.

Experimental

Materials

L-Glutamine and Hepes [2-(*N*-2-hydroxyethyl)-piperazin-*N'*-yl)ethanesulphonic acid] were products of Sigma Chemical Co., St. Louis, Mo., U.S.A. The fatty *n*-acyl-CoA derivatives were obtained from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A.

Methods

Phosphate-activated glutaminase from pig kidney and pig brain was purified as described previously (Kvamme *et al.*, 1970; Svenneby *et al.*, 1973). The second Tris-HCl-solubilized fraction [specific activity about 40 units (1 unit is defined as 1 μ mol of NH_3 formed/min per mg of protein)] was used in this work.

Glutaminase was assayed by measuring the amount of glutamate formed during 5 min at 23°C; unless otherwise stated, the pH was 7.0. The reaction mixture (0.5 ml) contained 40 mM-L-glutamine, 3 mM-EDTA, 15 mM-Hepes and 0.01 mg of protein. The enzyme reaction was stopped by the addition of 1 ml of cold (0°C) ethanol. Controls were treated in the same manner for the determination of non-enzymically hydrolysed glutamine, except that the enzyme was added after the addition of ethanol. Glutamate was isolated by paper chromatography and measured quantitatively as described previously (Kvamme & Torgner, 1974a).

Protein was determined by the method of Layne (1957).

Results

Effect of fatty acyl-CoA derivatives on glutaminase

The fatty acyl-CoA derivatives with five or more fatty acyl carbons that we have investigated exert dual actions on glutaminase (Fig. 1). Whether the acyl-CoA derivatives have activating or inhibiting effects on the enzyme depends both on the acyl-CoA concentration and on the concentration of phosphate and other anionic compounds, e.g. citric acid-cycle intermediates, in the assay. Thus phosphate and to a minor extent citric acid-cycle intermediates potentiate the activation and protect against inactivation by the acyl-CoA derivatives in question, which behave similarly to Bromothymol Blue in this respect, and differently from acetyl-CoA.

Valeryl-CoA (5 carbons) and octanoyl-CoA (8 carbons) which are the CoA derivatives with the shortest saturated fatty acyl chain in this experimental series, show only activating effects in the concentration range 0–0.10 mM (Fig. 1a and Table 1). The inhibiting effect first becomes visible for decanoyl-CoA (10 carbons) at the highest concentration used (Fig. 1b). When the fatty acyl chain contains more than 10 carbons, the fatty acyl-CoA derivatives activate only in the presence of high phosphate concentrations (25 mM). Moreover, the concentration range for activation shifts towards lower concentrations and becomes increasingly narrow (Figs. 1c, 1d and 2a). This is quantitatively expressed in Table 1 by the A_{50} and I_{50} values, which are the concentrations required to give 50% maximum activation and 50% inhibition of the maximum

activity respectively. Thus the A_{50} and I_{50} values in general decrease when the number of saturated fatty acyl carbons increases. The very low concentrations (5 μ M) of palmitoyl-CoA and stearoyl-CoA that are required to inhibit the enzyme activity by 50% in the presence of 7 mM-phosphate should be noted.

Brain glutaminase generally appears to be more susceptible to activation and less to inhibition by long-chain acyl-CoA derivatives than kidney glutaminase (Table 1; Figs. 2b and 3b), but the experiments have not been repeated a sufficient number of times to permit statistical evaluation. Thus for brain glutaminase the A_{50} of palmitoyl-CoA and stearoyl-CoA with low phosphate concentrations present is 2 μ M and 1 μ M respectively, whereas the kidney enzyme is inhibited at these concentrations (I_{50} approx. 5 μ M). However, there is no appreciable difference in the effect of Bromothymol Blue on the two enzymes (Table 1).

The unsaturated fatty acyl-CoA, crotonoyl-CoA, affects glutaminase similarly to valeryl-CoA. The unsaturated long-chain fatty acyl-CoA derivatives, oleoyl-CoA and linoleoyl-CoA, activate both brain and kidney glutaminase, even with low phosphate concentrations present (A_{50} 4–5 μ M) (Table 1; Figs. 3a and 3b). Among the physiological compounds tested, these CoA derivatives appear to have the greatest resemblance to Bromothymol Blue with respect to the effect on both kidney and brain glutaminase.

By increasing the pH of the incubation medium from 7.0 to 8.0 the activating power of acyl-CoA derivatives becomes considerably more pronounced, and inhibition occurs at higher concentrations (Figs. 2c, 4a and 4b). Thus at pH 8.0 and with 7 mM or less phosphate present palmitoyl-CoA and stearoyl-CoA activate kidney glutaminase in the lower part of the concentration range tested (Fig. 2c), whereas at pH 7.0 they inhibit the enzyme under these conditions (Fig. 2a).

Effect of fatty acids on glutaminase

The saturated fatty acids with chain lengths up to 10 carbon atoms have no effect on glutaminase, as shown in Table 1 for the kidney enzyme. Saturated fatty acids with 12–14 carbon atoms inhibit glutaminase, but their I_{50} values are much higher than the values for the corresponding CoA derivatives, indicating that the latter compounds are more powerful inhibitors (Table 1). It should be noted that palmitic acid and stearic acid have no effect on glutaminase, in contrast with their CoA derivatives.

On the other hand, the concentration-activity plots of long-chain unsaturated fatty acids are of the Bromothymol Blue type, although their activating power is less pronounced than that of their

Fig. 1. Effect of saturated fatty acyl-CoA derivatives on pig kidney glutaminase

Glutaminase was assayed at pH 7.0 and the experimental conditions were as in Table 1. Variable ligand: (a) valeryl-CoA; (b) decanoyl-CoA; (c) dodecanoyl(lauryl)-CoA; (d) tetradecanoyl(myristyl)-CoA. Inset (A) and (B), double-reciprocal plots of enzyme activity versus substrate concentration with 7 mM-phosphate added. $\square$, No addition; Δ , 7 mM-phosphate added; $\circ$, 25 mM-phosphate added.

CoA derivatives (Fig. 3c and Table 1). Their I_{50} values are, however, similar to those of their CoA derivatives. It is of interest that palmitoyl-carnitine

activates glutaminase slightly at high concentrations (100 μM) only, whereas lower concentrations have no effect.

Table 1. Constants related to the activation and inhibition of pig kidney and brain glutaminase by fatty acyl-CoA derivatives and their parent fatty acids

The reaction mixture (0.5 ml) contained (sodium salts) 40 mM-L-glutamine, 15 mM-Hepes, 3 mM-EDTA and 0.01 mg of protein. Fatty acyl-CoA derivatives, fatty acids or Bromothymol Blue were added in various concentrations (1–100 μ M). Potassium phosphate was added as indicated. Glutamate formed was determined by chromatographic technique after incubation for 5 min at 23°C and pH 7.0. Maximum activation is expressed as a ratio of maximum activity with activator added (acyl-CoA derivative or fatty acid) to the activity without any addition. A_{50} is the concentration of activator required to give 50% maximum activation. I_{50} is the concentration of inhibitor required to give 50% inhibition of maximum activity. A_{50} and I_{50} were estimated graphically. The terms A_{50} and I_{50} are used instead of K_A and K_I , because true maximum activity cannot be determined under conditions where the activator also acts as an inhibitor. n.c., Not calculated. The experiments showing the effects of palmitoyl-CoA and stearoyl-CoA on kidney glutaminase were done four times and the average and range are recorded in the Table. Otherwise each experiment was done twice and the variation between parallels was within $\pm 15\%$ of the average.

	Phosphate (mM) ...	Maximum activation (fold)			$10^2 \times A_{50}$ (mM)			$10^2 \times I_{50}$ (mM)		
		0	7	25	0	7	25	0	7	25
1. Acyl-CoA derivatives in kidney	Crotonoyl-CoA	1.3	1.3	1.9	2.0	2.0	2.0	—	—	—
	Valeryl-CoA	1.3	1.6	1.8	3.0	2.0	2.0	—	—	—
	Octanoyl-CoA	1.0	1.2	2.5	—	n.c.	5.0	—	—	—
	Decanoyl-CoA	1.5	2.2	3.5	3.0	3.0	3.0	18.0	18.0	18.0
	Dodecanoyl-CoA	—	—	2.0	—	—	1.0	0.2	6.0	12.0
	Tridecanoyl-CoA	—	—	1.7	—	—	0.4	0.5	0.6	7.0
	Tetradecanoyl-CoA	—	1.2	4.0	—	n.c.	0.5	1.5	1.5	4.0
	Palmitoyl-CoA	—	—	1.9	—	—	0.2	0.5	0.5	1.0
				(1.7–2.0)			(0.15–0.20)	(0.5–0.7)	(0.5–0.6)	(1.0–1.1)
	Stearoyl-CoA	—	—	1.3	—	—	0.05	0.4	0.5	0.9
				(1.3–1.4)			(0.03–0.05)	(0.4–0.5)	(0.5–0.5)	(0.7–1.0)
	Oleoyl-CoA	1.2	1.6	2.5	n.c.	0.5	0.5	3.0	3.0	3.0
	Linoleoyl-CoA	1.7	2.3	3.6	0.4	0.4	0.2	4.5	4.5	7.0
	Bromothymol Blue	5.0	4.0	3.4	1.5	1.5	1.5	20.0	16.0	13.0
2. Fatty acids in kidney	Crotonic acid	1.3	1.1	1.0	3.0	—	—	—	—	—
	Valeric acid	1.0	1.0	1.0	—	—	—	—	—	—
	Octanoic acid	1.0	1.0	1.0	—	—	—	—	—	—
	Decanoic acid	1.0	1.0	1.0	—	—	—	—	—	—
	Dodecanoic acid	—	—	1.2	—	—	n.c.	10.0	12.0	—
	Tridecanoic acid	—	—	1.0	—	—	—	4.0	7.0	—
	Tetradecanoic acid	—	—	1.0	—	—	—	5.0	14.0	—
	Palmitic acid	1.0	1.0	1.0	—	—	—	—	—	—
	Stearic acid	1.0	1.0	1.0	—	—	—	—	—	—
	Oleic acid	—	1.2	2.2	—	n.c.	0.01	3.0	3.0	3.5
	Linoleic acid	—	—	1.2	—	—	0.01	2.7	3.7	3.5
3. Acyl-CoA derivatives in brain	Decanoyl-CoA	1.7	3.5	2.5	1.0	1.5	3.5	—	—	—
	Palmitoyl-CoA	1.7	1.5	2.8	0.2	0.2	0.2	1.0	1.0	1.3
	Stearoyl-CoA	1.4	1.2	1.3	0.1	0.1	0.1	1.0	1.0	1.5
	Oleoyl-CoA	2.5	2.0	3.4	0.4	0.4	0.6	8.0	8.0	9.0
	Bromothymol Blue	6.0	5.0	6.0	3.0	3.0	3.0	18.0	15.0	15.0

Interactions between fatty acyl-CoA derivatives and other activators

As described above, phosphate potentiates the activation by long-chain acyl-CoA derivatives. Citrate and α -oxoglutarate have similar effects which are less pronounced than for phosphate. Fig. 4(b) demonstrates the potentiation by citrate compared with that by phosphate of the activation by linoleoyl-CoA.

Plots of enzyme activity versus acyl-CoA concentration, however, are more or less sigmoidal, indi-

cating positive co-operativity at low concentrations (Fig. 4b). Inverse plots of enzyme activity versus acyl-CoA concentration, however, have an upwards-convex form (insets A and B, Figs. 1a and 1b), which is most pronounced at high concentrations, and may thus be due to the dual effects of these compounds on glutaminase. Moreover, plots of enzyme activity versus phosphate concentration are sigmoidal when fatty acyl-CoA derivatives are present. The double-inverse plots are in this case upwards concave. In the presence of different activating concentrations

Fig. 2. Effect of palmitoyl-CoA on pig kidney and brain glutaminase

(a) Pig kidney glutaminase, pH 7.0; (b) pig brain glutaminase, pH 7.0; (c) pig kidney glutaminase, pH 8.0. The experimental conditions were otherwise as in Table 1. $\square$, No addition; Δ , 7 mM-phosphate added; $\circ$, 25 mM-phosphate added.

Fig. 3. Effect of oleoyl-CoA and oleic acid on pig kidney and brain glutaminase

Glutaminase was assayed at pH 7.0 and the experimental conditions were as in Table 1. (a) Pig kidney glutaminase, oleoyl-CoA variable ligand; (b) pig brain glutaminase, oleoyl-CoA variable ligand; (c) pig kidney glutaminase, oleic acid variable ligand. $\square$, No addition; Δ , 7 mM-phosphate added; $\circ$, 25 mM-phosphate added.

Fig. 4. Effect of oleoyl-CoA and linoleoyl-CoA when pH increases from 7.0 to 8.0 on pig kidney glutaminase

Except for different pH values, the experimental conditions were as in Table 1. (a) Variable ligand oleoyl-CoA, pH 8.0; $\square$, no addition; Δ , 7 mM-phosphate added; $\circ$, 25 mM-phosphate added. (b) Variable ligand linoleoyl-CoA, pH 8.0; $\square$, no addition; Δ , 7 mM-phosphate added; $\bullet$, 25 mM-phosphate added. (c) Double-reciprocal plots of enzyme activity versus phosphate concentration at pH 7.0; $\square$, no addition; $\circ$, 0.01 mM-linoleoyl-CoA added; Δ , 0.08 mM-linoleoyl-CoA added.

of fatty acyl-CoA (Fig. 4c) as well as inhibiting ones (Kvamme & Torgner, 1974b), the plots intersect at one point on the ordinate, suggesting competition between phosphate and fatty acyl-CoA derivatives.

Reversibility of the inhibition of glutaminase by acyl-CoA derivatives and unsaturated fatty acids

As demonstrated in the Figures, phosphate counteracts the inhibition of glutaminase by acyl-CoA derivatives and the unsaturated fatty acids oleic acid and linoleic acid, but it cannot be decided from these data whether phosphate reverses the inhibition or if it only protects against irreversible inactivation. In the latter case, the inhibition would be expected to be of minor physiological importance. However, the apparent competition between phosphate and fatty acyl-CoA derivatives, which is described above, suggests that the effect of fatty acyl-CoA derivatives on glutaminase is reversible. To investigate this further glutaminase was preincubated with inhibiting concentrations of the compounds and with no phosphate added, followed by assay with and without the addition of excess of phosphate. Table 2 demonstrates that the inhibition by low concen-

trations of fatty acyl-CoA derivatives or by unsaturated fatty acids is reversed by phosphate, whereas higher concentrations inhibit the enzyme irreversibly. However, phosphate is much more effective when added together with the inhibitor instead of after it, so that phosphate appears not only to reverse the inhibition, but also to protect against inactivation. No restoration of activity was possible, e.g. by extensive dialysis against Hepes-EDTA buffer, once the enzyme became inactivated.

It has been reported that inhibition by palmitoyl-CoA of some enzymes is reversed by their substrates, e.g. glucose 6-phosphate reverses the inhibition of glucose 6-phosphate dehydrogenase (Takata & Pogell, 1966). As shown in Table 3, glutamine does not prevent the inhibition of glutaminase by palmitoyl-CoA or stearoyl-CoA. Even if the glutamine concentration is increased from 0.5 mM to 20.0 mM, the percentage inhibition of glutaminase is unchanged, e.g. about 60% in the presence of 0.01 mM-palmitoyl-CoA. In accordance with this, it has previously been found that the inhibition by palmitoyl-CoA and stearoyl-CoA is non-competitive with glutamine (Kvamme & Torgner, 1974b).

Reversibility experiments, as described in this section, revealed no difference between kidney and brain glutaminase.

Table 2. *Reversal by phosphate of the inhibition of glutaminase by fatty acyl-CoA derivatives and unsaturated fatty acids*

Pig kidney glutaminase was preincubated for 5 min at 23°C and pH 7.0 with fatty acyl-CoA derivatives or fatty acids in concentrations as indicated in the Table. The preincubation mixture contained also 20 mM-Hepes and 4 mM-EDTA. Thereafter the enzyme was assayed as described in Table 1 (5 min at 23°C and pH 7.0) with and without the addition of phosphate. Water was added to the controls which did not contain phosphate. By these additions the enzyme was diluted 10% and by the subsequent addition of glutaminase a total dilution of 25% resulted, which, however, did not affect the results. The experiments were repeated three times and the results are expressed as the ratio of the activity with inhibitor present to that without.

	Phosphate (mM)	Acyl-CoA (mM) ...	Ratio		
			0.005	0.01	0.02
Palmitoyl-CoA	0		0.65	0.44	0
	50		1.18	1.05	0
Stearoyl-CoA	0		0.17	0	0
	50		0.33	0	0
Oleoyl-CoA	0		0.65	0.08	0
	150		1.02	0.11	0
		Fatty acids (mM) ...	0.0150	0.0250	0.0500
Oleic acid	0		0.75	0.37	0
	150		1.11	0.80	0.10
Linoleic acid	0		1.02	0.43	0.31
	150		1.05	0.91	0.26

Table 3. *Non-protective effect by the substrate glutamine of the inhibition of glutaminase by acyl-CoA derivatives*

Pig kidney glutaminase was incubated with 10 mM-phosphate, and with glutamine, palmitoyl-CoA and stearoyl-CoA as indicated in the Table. The experimental conditions were otherwise as in Table 1. The results are expressed as the ratio of the activity with inhibitor present to that without.

	Glutamine (mM)	Acyl-CoA (mM) ...	Ratio			
			0.0025	0.0050	0.0075	0.0100
Palmitoyl-CoA	0.5		1.03	1.09	0.92	0.41
	1.0		1.09	1.08	0.84	0.45
	2.0		1.02	1.04	0.80	0.42
	5.0		1.02	1.03	0.75	0.39
	20.0		1.06	1.11	0.86	0.45
Stearoyl-CoA	0.5		0.95	0.87	0.67	0.38
	1.0		0.97	0.87	0.61	0.39
	2.0		1.03	0.88	0.65	0.29
	5.0		1.02	0.86	0.56	0.27
	20.0		1.08	0.85	0.57	0.30

Discussion

Palmitoyl-CoA in concentrations 0.1–10 μ M has previously been found to irreversibly inhibit many mammalian enzymes, e.g. glutamate dehydrogenase, malate dehydrogenase, fumarase, α -glycerophosphate dehydrogenase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Takata & Pogell, 1966), as well as yeast and bacterial fatty acid synthetases (Knoche *et al.*, 1973). However, the inhibition of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase was competitive and reversible with respect to their substrates, so that the inhibition may have some physiological importance in the regulation of the activity of these

enzymes. Moreover, palmitoyl-CoA inhibits mitochondrial adenine nucleotide translocase (Pande & Blanchaer, 1971) and long-chain acyl-CoA synthetase from rat liver (Pande, 1973) in a fully reversible manner. Apart from the effects of palmitoyl-CoA, little information is available about the effects of other long-chain acyl-CoA derivatives on mammalian enzyme activities.

Activation of mammalian enzymes by long-chain fatty acyl-CoA derivatives has, to our knowledge, not been described previously, but activation of phosphoenolpyruvate carboxykinase of *Escherichia coli* by free fatty acids such as laurate and oleate, and by their CoA derivatives, has been found

(Izui *et al.*, 1970). Thus it is noteworthy that mitochondrial glutaminase is strongly activated by many fatty acyl-CoA derivatives, both saturated and unsaturated, in the lowest concentration range in which they show any effect, and that the activation is potentiated by other anions, such as phosphate. At higher concentrations, but within a very narrow range, the long-chain fatty acyl-CoA derivatives that we have investigated, become inhibitors, and the inhibition is reversed by phosphate. At still higher concentrations, the inhibition becomes irreversible, but this effect is probably of little physiological interest.

Fatty acyl-CoA derivatives with 5–18 fatty acyl carbon atoms also affect phosphate-activated glutaminase from kidney and brain differently from acetyl-CoA, because phosphate or citrate in concentrations higher than 10mM and 100mM respectively abolish the activation by acetyl-CoA (Kvamme & Torgner, 1974a). Since it is unlikely that the phosphate concentration in the mitochondrial matrix space ever falls below 5mM, and acetyl-CoA is increasingly effective when the substrate concentration decreases, it may thus secure a certain minimum glutaminase activity at phosphate concentrations in the range 5–10mM and at low glutamine concentrations.

Saturated fatty acyl-CoA derivatives with 5–18 fatty acyl carbons become more potent inhibitors with increasing numbers of acyl carbons, and more potent activators when the acyl chain becomes shorter. Thus the activation may be due to interaction with hydrophilic groups on the enzyme, whereas the increasing hydrophobicity of the longer fatty acyl chains may cause the inhibition. In keeping with this, the unsaturated acyl-CoA derivatives with 18 fatty acyl carbons, oleoyl-CoA and linoleoyl-CoA, are more potent activators and less potent inhibitors than the corresponding saturated derivative, palmitoyl-CoA.

Similarly to Bromothymol Blue (Kvamme & Torgner, 1974a), the 5–18-carbon fatty acyl-CoA derivatives, both in activating (Fig. 4c) and inhibiting concentrations (Kvamme & Torgner, 1974b), affect mainly the apparent K_m value for phosphate with little or no change in the $V_{max.}$, and they appear thus to affect the binding of anions to the anionic activating sites on glutaminase. Moreover, the affinity of fatty acyl-CoA derivatives for inhibiting hydrophobic binding sites on the enzyme seems to be lower than that for the neighbouring activating hydrophilic binding sites. In accordance with this, the acyl-CoA derivatives appear not to affect the binding sites for the substrate glutamine, because glutamine does not protect against inhibition by these compounds.

With respect to the physiological importance of the effects of fatty acyl-CoA derivatives of glutaminase,

no conclusions can, of course, be drawn from experiments *in vitro* alone. Moreover, Morei *et al.* (1974) emphasize the high, and unspecific, acceptance capacity of biological membranes for acyl-CoA derivatives. *In vivo* there are also problems of compartmentalization and local concentrations to be taken into account. However, the reversible effects on glutaminase of acyl-CoA derivatives, as described in this and a previous paper (Kvamme & Torgner, 1974a), open up the possibility of a very refined metabolic regulation, linking NH_3 production and amino acid metabolism to lipid oxidation. Apart from the interaction of acyl-CoA derivatives with, for example, phosphate or citrate, regulation of glutaminase activity is also possible by increasing the chain length of the fatty acyl-CoA derivatives, by splitting off the CoA or by replacing it with carnitine, by a change in H^+ concentration and by unsaturation of the acyl moiety.

Further, it is known that the intramitochondrial concentrations of long-chain fatty acyl-CoA derivatives undergo great variations, depending both on the nutritional state of the animal and on the organ in question. Thus the long-chain fatty acyl-CoA concentrations of rat liver mitochondria vary in the range 0.1–0.5nmol/mg of protein (Garland *et al.*, 1965). If confined to the mitochondrial matrix, like phosphate-activated glutaminase (Crompton *et al.*, 1973), local concentrations may be well within the effective range for these derivatives.

Preliminary experiments in our laboratory show that palmitoyl-carnitine exerts a concentration-dependent effect on glutaminase in intact pig kidney mitochondria, which is not seen in experiments with the purified enzyme, indicating that acyl-CoA derivatives formed from palmitoyl-carnitine are permitted to enter the mitochondrial matrix space where glutaminase is localized.

It should be noted that free fatty acids are major fuels of the kidney (Gold & Spitzer, 1964; Nieth & Schollmeyer, 1966). In chronic metabolic acidosis increased amounts of NH_3 are formed from glutamine (Shalhoub *et al.*, 1963; Owen & Robinson, 1963) and an activation of glutaminase has been discussed (Rector *et al.*, 1965; Damian & Pitts, 1970). It has also been reported that glutaminase becomes more sensitive to activation by phosphate under these conditions. At any rate, the well-known increased concentrations of acyl-CoA derivatives in diabetic acidosis may explain the activation of glutaminase in this type of acidosis.

This work was supported by grants from the Norwegian Research Council for Science and the Humanities and from Brukseier Jonn Nilsen og hustru Maja Jonn-Nilsen's legat. We are indebted to Mrs. Berit Rinde Juel and Mrs. Anne-Lovise Naess-Halvorsen for their skilful technical assistance.

References

- Crompton, M., McGivan, J. D. & Chappell, J. B. (1973) *Biochem. J.* **132**, 27–34
- Damian, A. C. & Pitts, R. F. (1970) *Am. J. Physiol.* **218**, 1249–1255
- Garland, P. B., Shepherd, D. & Yates, D. W. (1965) *Biochem. J.* **97**, 587–594
- Gold, M. & Spitzer, J. J. (1964) *Am. J. Physiol.* **206**, 153–158
- Izui, K., Yoshinaga, T., Morikawa, M. & Katsuki, H. (1970) *Biochem. Biophys. Res. Commun.* **40**, 949–956
- Knoche, H., Esders, T. W., Koths, K. & Bloch, K. (1973) *J. Biol. Chem.* **248**, 2317–2322
- Kvamme, E. & Torgner, I. Aa. (1974a) *Biochem. J.* **137**, 525–530
- Kvamme, E. & Torgner, I. Aa. (1974b) *FEBS Lett.* **47**, 244–247
- Kvamme, E., Tveit, B. & Svenneby, G. (1965) *Biochem. Biophys. Res. Commun.* **20**, 566–572
- Kvamme, E., Tveit, B. & Svenneby, G. (1970) *J. Biol. Chem.* **245**, 1871–1877
- Layne, E. (1957) *Methods Enzymol.* **3**, 447–454
- Morei, F., Laguain, G., Lunardi, J., Duszynski, J. & Vignais, P. V. (1974) *FEBS Lett.* **39**, 133–138
- Nieth, H. & Schollmeyer, P. (1966) *Nature (London)* **209**, 1244–1245
- Owen, E. E. & Robinson, R. R. (1963) *J. Clin. Invest.* **42**, 263–276
- Pande, S. V. (1973) *Biochim. Biophys. Acta* **306**, 15–20
- Pande, S. V. & Blanchaer, M. C. (1971) *J. Biol. Chem.* **246**, 402–411
- Rector, F. C., Seldin, D. W. & Copenhaver, J. H. (1965) *J. Clin. Invest.* **34**, 20–26
- Shalhoub, R., Weber, W., Glabman, S., Canessa-Fischer, M., Klein, J., Deltaas, J. & Pitts, R. F. (1963) *Am. J. Physiol.* **204**, 181–186
- Svenneby, G. (1970) *J. Neurochem.* **17**, 1591–1599
- Svenneby, G. (1972) *J. Neurochem.* **19**, 165–174
- Svenneby, G., Tveit, B. & Kvamme, E. (1970) *J. Biol. Chem.* **245**, 1878–1882
- Svenneby, G., Torgner, I. Aa. & Kvamme, E. (1973) *J. Neurochem.* **20**, 1217–1224
- Takata, K. & Pogell, B. M. (1966) *J. Biol. Chem.* **241**, 720–726
- Tveit, B., Svenneby, G. & Kvamme, E. (1970) *Eur. J. Biochem.* **14**, 337–344
- Weil-Malherbe, H. (1972) *J. Neurochem.* **19**, 2257–2267
- Weil-Malherbe, H. & Beall, G. D. (1970) *J. Neurochem.* **17**, 1101–1103