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Effects of adrenaline on ketogenesis from long- and medium-chain fatty acids in starved rats

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1. Injection of adrenaline into 24h-starved rats caused a 69% decrease in blood [ketone-body] (3-hydroxybutyrate plus acetoacetate), accompanied by a decreased [3-hydroxybutyrate]/[acetoacetate] ratio. Blood [glucose] and [lactate] increased, but [alanine] was unchanged. 2. Adrenaline also decreased [ketone-body] after intragastric feeding of both long- and medium-chain triacylglycerol. The latter decrease was observed after suppression of lipolysis with 5-methylpyrazole-3-carboxylic acid, indicating that the antiketogenic action of adrenaline was not dependent on the chain length of the precursor fatty acid. 3. The actions of adrenaline to decrease blood [ketone-body] and to increase blood [glucose] were not observed after administration of 3-mercaptopicolinate, an inhibitor of gluconeogenesis. This suggests that these effects of the hormone are related. 4. The possible clinical significance of the results is discussed with reference to the restricted ketosis often observed after surgical or orthopaedic injury.

The increased rate of ketogenesis observed in the fed-to-starved transition has been attributed to a decreased plasma insulin concentration secondary to hypoglycaemia [reviewed by Williamson & Whitelaw (1978) and McGarry & Foster (1980)]. Hypoinsulinaemia increases adipose-tissue lipolysis and thus the supply of long-chain fatty acids (the major substrates for ketogenesis) to the liver. In addition, a fall in blood concentration of insulin relative to that of glucagon increases hepatic oxidation of long-chain fatty acids and decreases their esterification to triacylglycerol. Here a major control point is the carnitine acyltransferase system, which is required for transfer of long-chain fatty acyl-CoA into the mitochondrion for oxidation. One regulatory mechanism is the inhibition of carnitine acyltransferase I, the first enzyme of the carnitine shuttle, by malonyl-CoA, an intermediate of lipogenesis (see McGarry & Foster, 1980). Thus when lipogenesis is depressed, the concomitant lowering of the malonyl-CoA concentration will divert fatty acids into oxidative pathways.

In stress, as in starvation, hepatic fatty acid supply is increased as a result of fat mobilization. Circulating insulin concentrations are decreased (Russell *et al.*, 1975) and glucagon and catecholamine concentrations are elevated (Hallberg & Orö, 1965; see Traynor & Hall, 1981). Despite the lowered insulin/glucagon concentration ratio and increased fatty acid supply, marked ketosis is not a characteristic of stress states, especially those associated with surgery or injury (Smith *et al.*, 1975; Wedge *et al.*, 1976). The results of infusion studies (e.g. Schade & Eaton, 1977, 1979) have suggested that the catecholamines are ketogenic *in vivo*, acting mainly by stimulating lipolysis and thus increasing hepatic fatty acid supply (for review, see Alberti *et al.*, 1978). The effects of the catecholamines have not, however, been extensively investigated in the starved state, where adipose-tissue lipolysis is already stimulated. In this regard, surgical patients are often starved pre- and post-operatively, and food is not usually administered during the first 24h after orthopaedic trauma (see, e.g., Wedge *et al.*, 1976). The present paper reports that subcutaneous injection of adrenaline, in contrast with the expected result, decreases blood ketone-body concentrations in starved rats. We suggest that this effect of adrenaline may explain the low blood ketone-body concentrations often found in patients post-operatively or after severe injury. If this is the case, the accelerated protein loss observed in such patients

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(O'Donnell *et al.*, 1976) may be a direct consequence of stress-induced increases in circulating adrenaline.

Experimental

Animals

Female Albino Wistar rats (150–180g) were subjected to a 12h-light/12h-dark cycle, the light period starting at 08:00h, and, unless otherwise stated, were used after 24h starvation in grid-bottomed cages. Fed rats were allowed free access to standard rodent diet [BP Nutrition (U.K.) Ltd., Witham, Essex, U.K.]. Water was supplied *ad libitum*.

Studies in vivo

Experiments were started between 09:00 and 09:30h. Glucose (2mmol/100g body wt.; 2M solution) was administered intragastrically as described by Sugden *et al.* (1981), and long-chain triacylglycerol (1.5ml) or medium-chain triacylglycerol (0.5 or 1.5ml) was administered similarly. Controls were intubated with water (0.5 or 1.5ml). Rats were killed 2h after intragastric feeding.

Administration of drugs and hormones

Heparin (200i.u.) was injected into the femoral vein under diethyl ether anaesthesia immediately before intubation. Insulin (2 units) and adrenaline (20μg/100g body wt.) were injected subcutaneously 0.25h (insulin) or 1h (adrenaline) after intragastric feeding, i.e. 1.75h or 1h before the rats were killed. Sodium 3-mercaptopicolinate (20mg/100g body wt.) in 0.5ml of 0.9% NaCl, pH7.4, and 5-methylpyrazole-3-carboxylic acid (0.66mg/100g body wt.) in 0.2ml of 0.9% NaCl, pH7.4, were injected intraperitoneally at the time of intubation. A booster dose of 5-methylpyrazole-3-carboxylic acid was given 1h after intubation. In controls, 0.9% NaCl was injected instead of hormones or drugs.

Measurement of rates of hepatic lipogenesis

Rats were injected intraperitoneally with ^3H_2O (5mCi; 0.5ml in 0.9% NaCl) 1h after intubation, anaesthetized 50min later (sodium pentobarbital; 6mg/100g body wt.) and dissected at 1h as described by Agius & Williamson (1980). The incorporation of ^3H into lipid was measured by the method of Stansbie *et al.* (1976). An arterial blood sample was withdrawn at this time for determination of plasma ^3H_2O specific radioactivity and blood metabolite concentrations.

Determination of metabolites

Arterial blood samples were treated with 10% (v/v) $HClO_4$ (2.0ml/0.5ml of whole blood), and D-glucose (Slein, 1963), alanine (Williamson, 1974), lactate (Hohorst *et al.*, 1959) and 3-hydroxybutyrate and acetoacetate (Williamson *et al.*, 1962) determined in KOH-neutralized $HClO_4$ extracts. Blood ketone-body concentration refers to the sum of the concentrations of 3-hydroxybutyrate and acetoacetate. Acetone was not measured. Insulin was assayed in plasma by a double-antibody method (Soeldner & Slone, 1965). Statistical significance of differences was assessed with Student's unpaired *t* test. Results are given as means $\pm$ S.E.M., with the numbers of observations given in parentheses.

Materials

Enzymes and coenzymes were from BCL Ltd., Lewes, East Sussex, U.K. ^3H_2O was from Amersham International, Amersham, Bucks., U.K. Long-chain triacylglycerol (glycerol trioleate) was from BDH Chemicals, Poole, Dorset, U.K. Medium-chain triacylglycerol (a mixture of C_6–C_{12} fatty acids with at least 95% C_8 and C_{10}) was from Cow and Gate, Trowbridge, Wilts., U.K. Insulin (Isophane) was from Nordisk Laboratories, Copenhagen, Denmark. Adrenaline injection BP (in 0.9% NaCl as acid tartrate, together with sodium metabisulphite as stabilizing agent) was from Antigen Ltd., Roscrea, Ireland. Heparin injection BP (Pabyrn) was from Paines and Byrne, Greenford, Middx., U.K. 5-Methylpyrazole-3-carboxylic acid was a gift from Upjohn Ltd., Crawley, West Sussex, U.K. 3-Mercaptopicolinic acid was a gift from Dr. N. W. DiTullio, Smith, Kline and French Laboratories, Philadelphia, PA, U.S.A.

Results

Effects of adrenaline in fed and starved rats

Rats fed *ad libitum* had blood ketone-body concentrations of 0.15 ± 0.03 (4)μmol/ml (Table 1). Starvation of rats for 24h increased blood ketone bodies 5.8-fold ($P < 0.001$, Table 1) and decreased hepatic lipogenesis 3.9-fold from 21.9 ± 3.3 (4)μg-atoms of ^3H incorporated/h per g wet wt. of liver to 5.6 ± 1.3 (7)μg-atoms/h per g ($P < 0.001$). The increase in blood ketone-body concentration therefore may reflect both increased long-chain fatty acid supply to the liver and increased entry of long-chain fatty acyl-CoA into the mitochondria for oxidation. Adrenaline injection of 24h-starved rats caused a marked (69%) decrease in blood ketone-body concentration (Table 1), but there was no increase in hepatic lipogenesis [+ adrenaline, 5.7 ± 0.4 (9)μg-atoms of ^3H incorporated/h per g wet wt.] This suggests that the decrease in ketone bodies is not due to inhibition of entry of fatty acyl-CoA into the mitochondrion by an increased hepatic malonyl-CoA concentration. The decreased ketone-body concentration was accompanied by a decrease in

Table 1. *Effects of adrenaline on blood ketone-body concentrations after triacylglycerol feeding*

For experimental details see the text. The values are means ± S.E.M. for the numbers of rats shown in parentheses. Values that are significantly different from each other are shown: ***$P < 0.001$, *$P < 0.05$ for rats not injected with adrenaline (NaCl-injected) and rats injected with adrenaline in any one experimental group; †††$P < 0.001$, †$P < 0.05$ for differences between water-fed and triacylglycerol-fed rats.

Treatment of rats	Blood ketone bodies (μmol/ml)	
	NaCl-injected	Adrenaline-injected
Fed *ad libitum*		
+ Water	0.15 ± 0.03 (4)	0.16 ± 0.02 (4)
24 h-starved		
+ Water	0.87 ± 0.08 (7)	0.27 ± 0.03 (9)***
+ Medium-chain triacylglycerol (0.5 ml)	2.17 ± 0.20 (6)†††	0.71 ± 0.16 (5)***
+ Medium-chain triacylglycerol (1.5 ml)	3.06 ± 0.13 (6)†††	2.19 ± 0.36 (5)*
+ Long-chain triacylglycerol	1.12 ± 0.08 (6)†	0.17 ± 0.01 (5)***
+ Long-chain triacylglycerol + heparin	1.79 ± 0.10 (4)†††	0.57 ± 0.06 (5)***

[3-hydroxybutyrate]/[acetoacetate] from 2.8 ± 0.3 (7) to 1.7 ± 0.2 (9) ($P < 0.01$). This change in ratio indicates a more oxidized mitochondrial free NAD^+/NADH system. Adrenaline administration had no effect on hepatic lipogenesis (results not shown) or blood ketone-body concentrations (Table 1) in fed animals

Effects of adrenaline after triacylglycerol feeding

In contrast with long-chain fatty acids, medium- and short-chain fatty acids are not esterified (McGarry & Foster, 1971*a*) and do not require the carnitine shuttle for transfer to the mitochondrial matrix (see Williamson, 1979). To confirm that the hypoketonaemic action of adrenaline was not due to inhibition of the carnitine shuttle, we compared the effects of the hormone after a single intragastric load of either medium- or long-chain triacylglycerol.

Administration of long-chain triacylglycerol slightly increased the blood ketone-body concentration in 24 h-starved rats. A more significant (106%) elevation was found if heparin was given at the time of intragastric loading (Table 1). Blood ketone bodies were also increased by giving medium-chain triacylglycerol (Table 1). An intragastric load of 0.5 ml of medium-chain triacylglycerol produced blood ketone-body concentrations comparable with those found in rats treated with long-chain triacylglycerol plus heparin. If the volume of medium-chain triacylglycerol was 1.5 ml, higher blood concentrations of ketone bodies were achieved (Table 1). Injection of adrenaline into rats in every experimental group decreased the blood ketone-body concentration (Table 1), and the percentage decrease in concentration was similar if the rats were treated with water (i.e. ketone bodies were derived from endogenous long-chain fatty acids), 0.5 ml of medium-chain triacylglycerol, or long-chain triacylglycerol plus heparin (69%, 67% and 68% respectively; Table 1). The effect of adrenaline was less marked (28%) after administration of 1.5 ml of medium-chain triacylglycerol. It is important to stress that, when the initial concentrations of ketone bodies were comparable, the percentage decrease in ketone-body concentration was not dependent on whether the substrates for ketogenesis were long-chain or medium-chain fatty acids. These experiments therefore confirm that the effect of adrenaline to decrease blood ketone-body concentrations is not due to inhibition of carnitine acyltransferase. Moreover, since medium-chain fatty acids are not esterified, the decreases in blood ketone-body concentrations are not secondary to diversion of fatty acids into esterified products. Thus, either adrenaline inhibits ketogenesis by a mechanism which is independent of the chain-length of the precursor fatty acids, or adrenaline promotes the utilization of ketone bodies by peripheral tissues. In most situations the turnover of ketone bodies in the rat varies approximately in proportion to their circulating concentrations (see Robinson & Williamson, 1980). Thus a decrease in circulating ketone bodies is more likely to reflect a lower rate of ketogenesis than increased peripheral extraction.

Effects of adrenaline in the presence of 5-methylpyrazole-3-carboxylic acid

As ketone bodies inhibit lipolysis (Björntorp, 1966; Björntorp & Scherstén, 1967; see Robinson & Williamson, 1980), the high blood ketone-body concentrations produced by giving medium-chain triacylglycerol should suppress release of long-chain fatty acids from adipose tissue. However, to eliminate any chance that circulating ketone bodies observed after giving medium-chain triacylglycerol were derived not only from medium-chain fatty acids, produced by the intestinal cells and released into the portal blood stream (Senior, 1968), but also

from long-chain fatty acids released from endogenous fat stores, the experiments were repeated after suppression of lipolysis with 5-methylpyrazole-3-carboxylic acid (Axelrod *et al.*, 1979). Administration of 5-methylpyrazole-3-carboxylic acid to 24h-starved rats dramatically decreased plasma ketone bodies (Table 2). The concentration of ketone bodies observed after this treatment is the contribution of ketone bodies formed from endogenous long-chain fatty acids to total blood ketone bodies found after giving medium-chain triacylglycerol. Administration of 0.5ml of medium-chain triacylglycerol increased the blood ketone-body concentrations in 5-methylpyrazole-3-carboxylic acid-treated rats to 7 times the control (water + 5-methylpyrazole-3-carboxylic acid) value (Table 2). 5-Methylpyrazole-3-carboxylic acid did not decrease blood ketone bodies in rats treated with medium-chain triacylglycerol (Table 2), i.e., as expected, adipose-tissue lipolysis was inhibited after giving medium-chain triacylglycerol. Injection of adrenaline decreased blood ketone bodies in 5-methylpyrazole-3-carboxylic acid-treated rats given either water or medium-chain triacylglycerol (Table 2). The blood ketone-body concentration was decreased by 69%. This decrease was much greater than that which could be attributed to inhibition of ketogenesis from long-chain fatty acids (i.e. 1.51mM versus 0.12mM).

Hormone and metabolite interactions

Elevations of blood concentrations of lactate and glucose have been observed after surgical stress (Brandt *et al.*, 1976; Kehlet *et al.*, 1979) and have been attributed to increased circulating adrenaline concentrations (Christensen *et al.*, 1975; Brandt *et al.*, 1976; Kehlet *et al.*, 1979). Similarly we found that adrenaline injection into 24h-starved rats in each experimental group dramatically increased blood lactate and glucose (Table 3; also Table 5). Lactate is antiketogenic in perfused livers of starved rats and may decrease ketogenesis by increasing availability of glycerol 3-phosphate for esterification (McGarry & Foster, 1971*b*) or by acting as a precursor for oxaloacetate, thus increasing disposal of acetyl-CoA by the tricarboxylic acid cycle (Lehninger, 1946) and decreasing availability of acetyl-CoA for ketogenesis. Lactate exerts its major effect by the former mechanism or by increasing lipogenesis (McGarry & Foster, 1971*b*). Thus an increased lactate concentration would only be expected to inhibit ketogenesis from long-chain fatty acids. However, enhanced oxaloacetate formation should decrease ketone-body synthesis from both long-chain and medium-chain fatty acids, and this was what we observed. The blood concentration of alanine, which is hyperglycaemic, hyperlactaemic and antiketogenic in starved rats (Nosadini *et al.*, 1980), was not consistently increased by adrenaline injection (Table 3). Specifically there was no increase in alanine after adrenaline injection in 5-methylpyrazole-3-carboxylic acid-treated rats given 0.5ml of medium-chain triacylglycerol [control (4), $0.33 \pm 0.02\,\mu$mol/ml; plus adrenaline (5), $0.32 \pm 0.01\,\mu$mol/ml].

Increased blood glucose concentrations after injection of adrenaline into starved rats (Table 3; also Table 5) have also been observed by others (e.g. Yajima & Ui, 1974; Shikama & Ui, 1975). The hypoketonaemic effect of adrenaline might therefore be explained if insulin secretion was increased owing to the increased blood glucose, and insulin was directly responsible for the decreased ketone-body concentrations. This explanation is incorrect for the following reasons. Firstly, at the time of death, the plasma insulin concentration did not differ significantly between control (NaCl-injected) and adrenaline-injected rats, whereas control rats intubated with glucose had significantly ($P<0.001$) increased plasma insulin concentrations [control (3), 4.16 ± 1.25ng of insulin/ml of plasma; plus adrenaline (5), 6.27 ± 0.47ng/ml; plus glucose (3), 14.8 ± 0.9ng/ml]. Indeed it has been reported that the catecholamines inhibit insulin secretion (Porte *et*

Table 2. *Effects of adrenaline on ketogenesis from medium-chain triacylglycerol in the presence of 5-methylpyrazole-3-carboxylic acid in 24h-starved rats*

For experimental details see the text. The values are means ± S.E.M. for the numbers of rats shown in parentheses. Values that are significantly different from each other are shown: ***$P < 0.001$, **$P < 0.01$ for rats not injected with adrenaline (NaCl-injected) and rats injected with adrenaline; †††$P<0.001$, ††$P<0.01$ for differences between rats treated with water + 5-methylpyrazole-3-carboxylic acid, and rats in the other three groups.

Treatment of rats	Blood ketone bodies (μmol/ml)	
	NaCl-injected	Adrenaline-injected
Water	0.87 ± 0.08 (7)††	0.27 ± 0.03 (9)††
Water + 5-methylpyrazole-3-carboxylic acid	0.30 ± 0.06 (3)	0.18 ± 0.01 (3)**
Medium-chain triacylglycerol (0.5ml)	2.17 ± 0.20 (6)†††	0.71 ± 0.16 (5)†††
Medium-chain triacylglycerol (0.5ml) + 5-methylpyrazole-3-carboxylic acid	2.20 ± 0.06 (4)†††	0.69 ± 0.14 (5)***†††

Table 3. *Effects of adrenaline on blood metabolite concentrations after intragastric feeding of 24 h-starved rats*

For experimental details see the text. The values are means ± S.E.M. for the numbers of rats shown in parentheses. Values that are significantly different from those for the control (NaCl-injected) rats are shown: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Treatment of rats	Lactate (μmol/ml)		Glucose (μmol/ml)		Alanine (μmol/l)	
	NaCl-injected	Adrenaline-injected	NaCl-injected	Adrenaline-injected	NaCl-injected	Adrenaline-injected
Water	0.80 ± 0.13 (7)	1.96 ± 0.24 (7)***	6.21 ± 0.11 (7)	12.19 ± 0.57 (9)***	0.25 ± 0.02 (9)	0.20 ± 0.02 (10)
Medium-chain triacylglycerol (1.5 ml)	1.03 ± 0.19 (4)	2.04 ± 0.10 (5)***	5.32 ± 0.28 (6)	9.08 ± 0.28 (5)***	0.30 ± 0.02 (5)	0.24 ± 0.04 (5)
Long-chain triacylglycerol	0.67 ± 0.11 (6)	2.72 ± 0.22 (5)***	5.09 ± 0.08 (6)	11.05 ± 0.42 (5)***	0.20 ± 0.02 (4)	0.25 ± 0.01 (4)*
Long-chain triacylglycerol + heparin	0.61 ± 0.05 (4)	1.79 ± 0.31 (5)***	5.56 ± 0.08 (4)	9.60 ± 0.85 (5)**	0.18 ± 0.01 (4)	0.23 ± 0.04 (5)

al., 1966). Secondly, administration of insulin (which decreased blood ketone bodies in water-intubated 24 h-starved rats) did not decrease blood ketone bodies after an intragastric load of medium-chain triacylglycerol (Table 4), although insulin-induced hypoglycaemia was observed in all these animals. This observation *in vivo* is supported by the finding *in vitro* that insulin has no effect on ketogenesis from medium-chain fatty acids in hepatocytes prepared from starved animals (Witters & Trasko, 1979). Thus the effect of adrenaline on blood ketone-body concentrations in rats given medium-chain triacylglycerol is not mediated by an increased insulin concentration. Presumably the decreased blood ketone-body concentrations observed in the water-fed insulin-treated rats and in glucose-fed rats are secondary to inhibited release of long-chain fatty acids from adipose tissue, as rates of hepatic lipogenesis were not increased (Table 4).

Role of gluconeogenesis

To establish whether the action of adrenaline was secondary to the changes in blood glucose and blood lactate concentrations, the effect of the hormone was investigated after treatment of the rats with 3-mercaptopicolinic acid. This compound inhibits gluconeogenesis at the level of phosphoenolpyruvate carboxykinase (DiTullio *et al.*, 1974), and decreases blood glucose concentrations and increases blood lactate concentrations in 24 h-starved rats (Blackshear *et al.*, 1975). Our results are shown in Table 5. 3-Mercaptopicolinate increased blood lactate concentrations in rats intubated with water or medium-chain triacylglycerol. The blood lactate was not further increased by adrenaline administration. 3-Mercaptopicolinate also decreased blood glucose concentration in both water-intubated and triacylglycerol-intubated rats, and adrenaline failed to increase blood glucose in 3-mercaptopicolinate-treated animals. Although glucose 6-phosphatase has been detected in muscle (Surholt & Newsholme, 1981), and glucose may be directly released from muscle (Wicklmayr & Dietze, 1978), these results indicate that the increased glucose concentrations observed after adrenaline treatment are secondary to increased gluconeogenesis in that the glucose molecules entering the circulation are produced by gluconeogenesis rather than glycogenolysis. Others, from turnover studies, have come to the same conclusion (Shikama & Ui, 1975; see also Okajima & Ui, 1979). 3-Mercaptopicolinate slightly (though not significantly) decreased ketone-body formation in the starved (water-fed) animals (Table 5), and more dramatically decreased ketone-body concentrations in the rats given medium-chain triacylglycerol (Table 5). Adrenaline had little antiketogenic effect if the rats had been treated with 3-mercaptopicolinate (Table 5).

Table 4. *Effects of insulin on rates of hepatic lipogenesis in vivo and blood glucose and ketone-body concentrations after intragastric feeding of 24 h-starved rats*

For experimental details see the text. The values are means ± S.E.M. for the numbers of rats shown in parentheses. Significant differences between insulin-injected and control (NaCl-injected) rats are shown: ***$P < 0.001$. Lipogenesis is expressed as μg-atoms of ^3H incorporated into lipid/h per g wet wt. of liver.

	Lipogenesis		Blood ketone bodies (μmol/ml)		Blood glucose (μmol/ml)	
Treatment of rats	NaCl-injected	Insulin-injected	NaCl-injected	Insulin-injected	NaCl-injected	Insulin-injected
Water	5.2 ± 1.4 (6)	5.8 ± 0.6 (5)	0.81 ± 0.08 (9)	0.20 ± 0.01 (5)***	6.06 ± 0.10 (9)	1.51 ± 0.05 (6)***
Glucose	6.3 ± 0.9 (4)	5.1 ± 0.3 (6)	0.11 ± 0.04 (4)	0.09 ± 0.02 (6)	6.90 ± 0.70 (4)	2.03 ± 0.11 (6)***
Medium-chain triacylglycerol (1.5 ml)	6.9 ± 0.5 (6)	9.2 ± 1.6 (6)	3.06 ± 0.13 (6)	3.52 ± 0.31 (6)	5.32 ± 0.28 (6)	2.04 ± 0.20 (6)***

The blood ketone-body concentrations found after administration of adrenaline to water-fed rats were significantly higher if the rats had been treated with 3-mercaptopicolinate (Table 5). This would suggest that it is unlikely that adrenaline decreases blood ketone bodies via decreased provision of precursor to the liver (possibly via decreased hepatic blood flow; see Hems *et al.*, 1976). The inhibition of the hypoketonaemic action of adrenaline by 3-mercaptopicolinate would also tend to suggest that increased peripheral ketone-body utilization is not part of the mechanism. However, it should be stressed that our experiments do not directly measure hepatic ketogenesis, and, although to date the only known site of action of 3-mercaptopicolinate is inhibition of gluconeogenesis via inhibition of phosphoenolpyruvate carboxykinase, possible effects of adrenaline on hepatic fatty acid uptake or on peripheral ketone-body utilization cannot be excluded.

Discussion

Adrenaline lowers blood ketone-body concentrations when the substrates for ketogenesis are either medium-chain or long-chain fatty acids. Differences in the catabolism of medium-chain and long-chain fatty acids occur before the oxidative sequence, which occurs in the mitochondrial matrix (see above). Thus either adrenaline inhibits ketogenesis at an intramitochondrial site, or it increases ketone-body utilization. It is perhaps of interest that adrenaline-induced hyperglycaemia is associated with impaired glucose removal by peripheral tissues (Okajima & Ui, 1979) and it is possible that here ketone bodies (or fatty acids) are being used as alternative fuels. 3-Mercaptopicolinate blocks the effects of adrenaline both to increase blood glucose concentrations and to decrease blood ketone-body concentrations (Table 5). This suggests that these effects of adrenaline are related. One possibility is that the decrease in blood ketone-body concentration is secondary to an increase in gluconeogenesis. Gluconeogenesis from oxidized substrates depends on the availability of reducing equivalents in the cytosol (Williamson *et al.*, 1969), and adrenaline injection results in a more oxidized mitochondrial free $NAD^+/NADH$ system. Thus if adrenaline increases efflux of reducing equivalents from the mitochondrion, formation of 3-hydroxybutyrate from acetoacetate may be restricted. We have, however, no information regarding the oxidation state of the substrate used for gluconeogenesis in our experiments. Alternatively, to generate the ATP requirement for gluconeogenesis, the acetyl-CoA generated by β-oxidation may be completely oxidized to CO_2 and used as an energy substrate by the liver rather than being partially oxidized to ketone bodies. In support of this, adrenaline stimulates oxidation of [1-^{14}C]oleate to $^{14}CO_2$ in isolated rat (Sugden *et al.*, 1980) and mouse (Edwards *et al.*, 1981) hepatocytes. Increased oxidation to CO_2 of acetyl-CoA derived from fatty acids could be effected by increased tricarboxylic acid-cycle flux and/or stimulated respiratory-chain activity. In this regard, mitochondria prepared from hepatocytes from starved rats treated with the catecholamines show increased rates of ADP-dependent respiration and mitochondrial ATPase activity (Titheradge *et al.*, 1979; Titheradge & Hayes, 1980).

Clinical implications

After injury, patients have been divided into groups, namely those with, and those without, elevated blood concentrations of ketone bodies, and it was suggested that the patients in the hyperketonaemic group were less severely injured (Smith *et al.*, 1975; Wedge *et al.*, 1976). Other studies have indicated that again, after surgery, there is an inverse relationship between blood ketone-body concentration and severity of injury (Foster *et al.*, 1979). Severe trauma is characterized by an increase in the circulating glucagon/insulin concentration ratio (Lindsey *et al.*, 1974; Mequid *et al.*, 1972), and this was suggested to be responsible for the variation in the metabolic response to stress (Wedge *et al.*,

Table 5. *Effects of adrenaline on blood metabolite concentrations in the presence of 3-mercaptopicolinic acid in 24 h-starved rats*

For experimental details see the text. The values are means ± S.E.M. for numbers of rats shown in parentheses. Values that are significantly different from each other are shown: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ for differences between NaCl-injected and adrenaline-injected rats; †††$P < 0.001$, ††$P < 0.01$, †$P < 0.05$ for differences between rats treated with 3-mercaptopicolinate and rats not treated with 3-mercaptopicolinate in either the water-fed or the triacylglycerol-fed group.

Treatment of rats	Ketone bodies (μmol/ml)		Glucose (μmol/ml)		Lactate (μmol/ml)	
	NaCl-injected	Adrenaline-injected	NaCl-injected	Adrenaline-injected	NaCl-injected	Adrenaline-injected
Water	0.87 ± 0.08 (7)	0.27 ± 0.03 (9)	6.21 ± 0.11 (7)	12.19 ± 0.57 (9)	0.80 ± 0.13 (7)	1.96 ± 0.24 (7)
Water + 3-mercaptopicolinic acid	0.76 ± 0.04 (5)	0.61 ± 0.04 (5)*	1.55 ± 0.41 (5)†††	1.25 ± 0.15 (5)†††	1.94 ± 0.15 (5)†††	2.02 ± 0.31 (5)
Medium-chain triacylglycerol (0.5 ml)	2.17 ± 0.20 (6)	0.71 ± 0.16 (5)	5.26 ± 0.29 (6)	10.89 ± 0.33 (5)***	0.54 ± 0.08 (5)	2.19 ± 0.34 (5)**
Medium-chain triacylglycerol (0.5 ml) + 3-mercaptopicolinic acid	0.91 ± 0.07 (6)†††	0.80 ± 0.03 (5)	2.53 ± 0.04 (5)†††	2.05 ± 0.24 (5)†††	2.44 ± 0.16 (5)†††	2.78 ± 0.15 (5)

1976). However, although glucagon may enhance ketogenesis in insulin-deficient man (Alberti *et al.*, 1975), the hormone is relatively ineffective in the presence of even a low concentration of insulin (Schade & Eaton, 1975), and similar insulin concentrations have been observed in the more- and less-severely injured groups (Foster *et al.*, 1979). The present results therefore offer an alternative explanation for the differences in ketonaemia observed after trauma: patients responding to trauma with a transient stress-induced increase in circulating adrenaline concentrations would present in the normoketonaemic group. Indirect support for our suggestion is that patients in the normoketonaemic group have elevated blood lactate and glucose concentrations (Smith *et al.*, 1975; Wedge *et al.*, 1976; O'Donnell *et al.*, 1976), and we observe increases in blood glucose and lactate concentrations in rats treated with adrenaline. Similarly, increases in blood glucose and lactate concentrations [which are abolished by epidural analgesia and are therefore probably mediated by an increase in the circulating adrenaline concentration (Brandt *et al.*, 1976; Kehlet *et al.*, 1979)] are associated with decreased blood 3-hydroxybutyrate concentrations in the late post-operative period (Kehlet *et al.*, 1979). As the rate of nitrogen loss from the body after surgical operation, injury or serious infection is inversely related to the blood ketone-body concentration (O'Donnell *et al.*, 1976; Foster *et al.*, 1979), the absence of large increases of blood ketone-body concentrations in these patients may accelerate protein loss and delay complete recovery.

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References

Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480

Alberti, K. G. M. M., Christensen, N. J., Iversen, J. & Orskov, H. (1975) *Lancet* **i**, 1307–1311

Alberti, K. G. M. M., Johnston, D. G., Gill, A., Barnes, A. J. & Orskov, H. (1978) *Biochem. Soc. Symp.* **43**, 163–182

Axelrod, L., Lloyd-Jones, P. & Martin, D. (1979) *Diabetes* **28**, 651–657

Björntorp. P. (1966) *J. Lipid Res.* **7**, 621–626

Björntorp. P. & Scherstén, T. (1967) *Am. J. Physiol.* **212**, 683–684
Blackshear, P. J., Holloway, P. A. & Alberti, K. G. M. M. (1975) *Biochem. J.* **148**, 353–362
Brandt, M. R., Kehlet, H., Binder, C., Hagen, C. & McNeilly, A. S. (1976) *Clin. Endocrinol.* **5**, 107–114
Christensen, N. J., Alberti, K. G. M. M. & Brandsborg, O. (1975) *Eur. J. Clin. Invest.* **5**, 415–423
DiTullio, N. W., Berkoff, C. E., Blank, B., Kostos, V., Stack, E. J. & Saunders, H. L. (1974) *Biochem. J.* **138**, 387–394
Edwards, M. W., Cawthorne, M. A. & Williamson, D. H. (1981) *Biochem. J.* **198**, 239–242
Foster, K. J., Alberti, K. G. M. M., Binder, C., Hinks, L., Karran, S., Orskov, H., Smythe, P., Talbot, S. & Turnell, D. (1979) *Br. J. Surg.* **66**, 242–245
Hallberg, D. & Orö, L. (1965) *Acta Chir. Scand.* **178**, 281–290
Hems, D. A., Rodrigues, L. M. & Whitton, P. D. (1976) *Biochem. J.* **160**, 367–374
Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959) *Biochem. Z.* **332**, 18–46
Kehlet, H., Brandt, M. R., Prange Hansen, A. & Alberti, K. G. M. M. (1979) *Br. J. Surg.* **66**, 543–546
Lehninger, A. L. (1946) *J. Biol. Chem.* **164**, 291–306
Lindsey, A., Santeusanio, F., Braaten, J., Faloona, G. R. & Unger, R. H. (1974) *J. Am. Med. Assoc.* **227**, 757–761
McGarry, J. D. & Foster, D. W. (1971*a*) *J. Biol. Chem.* **246**, 1149–1159
McGarry, J. D. & Foster, D. W. (1971*b*) *J. Biol. Chem.* **246**, 6247–6253
McGarry, J. D. & Foster, D. W. (1980) *Annu. Rev. Biochem.* **49**, 395–420
Mequid, M. M., Brennen, M. F., Muller, W. A. & Aoki, T. T. (1972) *Lancet* **ii**, 1145
Nosadini, R., Datta, M., Hodson, A. & Alberti, K. G. M. M. (1980) *Biochem. J.* **190**, 323–332
O'Donnell, T. F., Clowes, G. H. A., Blackburn, G. L., Ryan, T., Benotti, P. N. & Miller, J. D. B. (1976) *Surgery* **80**, 192–200
Okajima, F. & Ui, M. (1979) *Biochem. J.* **182**, 585–592
Porte, D., Jr., Graber, A. L., Kuzuya, T. & Williams, R. H. (1966) *J. Clin. Invest.* **45**, 228–236
Robinson, A. M. & Williamson, D. H. (1980) *Physiol. Rev.* **60**, 143–187
Russell, R. C. G., Walker, C. J. & Bloom, S. R. (1975) *Br. Med. J.* **i**, 10–12
Schade, D. S. & Eaton, R. P. (1975) *Diabetes* **24**, 502–515
Schade, D. S. & Eaton, R. P. (1977) *Diabetes* **26**, 989–996
Schade, D. S. & Eaton, R. P. (1979) *Diabetes* **28**, 5–10
Senior, J. R. (1968) in *Medium-Chain Triglycerides* (Senior, J. R., ed.), pp. 3–6, University of Pennsylvania Press, Philadelphia
Shikama, H. & Ui, M. (1975) *Am. J. Physiol.* **229**, 962–966
Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
Smith, R., Fuller, D. J., Wedge, J. H., Williamson, D. H. & Alberti, K. G. M. M. (1975) *Lancet* **i**, 1–3
Soeldner, J. & Slone, D. (1965) *Diabetes* **14**, 771–779
Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
Sugden, M. C., Tordoff, A. F., Ilic, V. & Williamson, D. H. (1980) *FEBS Lett.* **120**, 80–84
Sugden, M. C., Watts, D. I. & Marshall, C. E. (1981) *Biosci. Rep.* **1**, 469–476
Surholt, B. & Newsholme, E. A. (1981) *Biochem. J.* **198**, 621–629
Titheradge, M. A. & Haynes, R. C., Jr. (1980) *Arch. Biochem. Biophys.* **201**, 44–55
Titheradge, M. A., Stringer, J. L. & Haynes, R. C., Jr. (1979) *Eur. J. Biochem.* **102**, 117–124
Traynor, C. & Hall, G. M. (1981) *Br. J. Anaesth.* **53**, 153–160
Wedge, J. H., De Campos, R., Kerr, A., Smith, R., Farrell, R., Ilic, V. & Williamson, D. H. (1976) *Clin. Sci. Mol. Med.* **50**, 393–399
Wicklmayr, M. & Dietze, G. (1978) *Eur. J. Clin. Invest.* **8**, 81–86
Williamson, D. H. (1974) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.) vol. 4, pp. 1679–1682, Academic Press, New York and London
Williamson, D. H. (1979) *Biochem. Soc. Trans.* **7**, 1313–1321
Williamson, D. H. & Whitelaw, E. (1978) *Biochem. Soc. Symp.* **43**, 137–161
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
Williamson, D. H., Veloso, D., Ellington, E. V. & Krebs, H. A. (1969) *Biochem. J.* **114**, 575–584
Witters, L. A. & Trasko, C. S. (1979) *Am. J. Physiol.* **237**, E23–E29
Yajima, M. & Ui, M. (1974) *Am. J. Physiol.* **227**, 1–7