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The Effect of Acetoacetate on Plasma Insulin Concentration

By R. A. HAWKINS, K. G. M. M. ALBERTI, C. R. S. HOUGHTON, D. H. WILLIAMSON and H. A. KREBS
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K.

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1. Sodium acetoacetate was infused into the inferior vena cava of fed rats, 48h-starved rats, and fed streptozotocin-diabetic rats treated with insulin. Arterial blood was obtained from a femoral artery catheter. 2. Acetoacetate infusion caused a fall in blood glucose concentration in fed rats from 6.16 to 5.11mM in 1h, whereas no change occurred in starved or fed–diabetic rats. 3. Plasma free fatty acids decreased within 10min, from 0.82 to 0.64mequiv./l in fed rats, 1.16 to 0.79mequiv./l in starved rats and 0.83 to 0.65mequiv./l in fed–diabetic rats. 4. At 10min the plasma concentration rose from 20 to 49.9 μunits/ml in fed unanaesthetized rats and from 6.4 to 18.5 μunits/ml in starved rats. There was no change in insulin concentration in the diabetic rats. 5. Nembutal-anaesthetized fed rats had a more marked increase in plasma insulin concentration, from 30 to 101 μunits/ml within 10min. 6. A fall in blood glucose concentration in fed rats and a decrease in free fatty acids in both fed and starved rats is to be expected as a consequence of the increase in plasma insulin. 7. The fall in the concentration of free fatty acids in diabetic rats may be due to a direct effect of ketone bodies on adipose tissue. A similar effect on free fatty acids could also be operative in normal fed or starved rats.

Experimental elevation of the blood concentrations of 3-hydroxybutyrate or acetoacetate in many species causes a decrease in the concentration of glucose and plasma free fatty acids (Neptune, 1956; Madison, Mebane, Unger & Lochner, 1964; Balasse, Couturier & Franckson, 1967; Björntorp & Scherstén, 1967; Senior & Loridan, 1968; Devecerski, Pierce & Frawley, 1968) and an increase in plasma insulin concentrations (Madison *et al.* 1964; Pi-Sunyer, Campbell & Hashim, 1970). The rise in the insulin concentrations, however, has not been observed under all conditions (Balasse *et al.* 1967; Balasse & Ooms, 1968; Devecerski *et al.* 1968; Loridan & Senior, 1970; Senior & Loridan, 1968).

In previous experiments, where circulating ketone-body concentrations of fed rats were raised by infusing acetoacetate, it was noted that the glucose concentration was lower than normal in fed rats (Hawkins, Williamson & Krebs, 1971). Further experiments showed that this observation could be accounted for by a stimulatory effect of acetoacetate on insulin secretion as described by the previous workers. Experiments described in the present paper show that infusion of acetoacetate into fed rats at rates sufficient to maintain ketone-body concentrations between 2 and 3mM or between 6 and 7mM uniformly resulted in elevated peripheral insulin concentrations. Other changes in metabolite concentrations were as expected in the presence of increased insulin concentrations.

MATERIALS AND METHODS

Rats. Female rats of the Wistar strain weighing 200–250g were used. They had free access to food unless otherwise indicated.

Reagents and analytical methods. ^{125}I-labelled insulin was obtained from Abbott Laboratories, North Chicago, Ill., U.S.A. Guinea-pig anti-insulin serum was a gift from Dr P. H. Wright, University of Indiana School of Medicine, Indianapolis, Ind., U.S.A. Goat anti-guinea-pig serum and protamine zinc insulin were obtained from Wellcome Laboratories, Beckenham, Kent, U.K. Streptozotocin was a gift of the Upjohn Co., Kalamazoo, Mich., U.S.A. Rat insulin was obtained from Novo, Copenhagen, Denmark.

Sodium acetoacetate for infusion was prepared from ethyl acetoacetate as described by Krebs & Eggleston (1941). It contained no ethanol when analysed by the method of Dickinson & Dalziel (1967).

Determination of metabolites and insulin. The following metabolites were determined in whole blood by standard enzymic methods: lactate and pyruvate (Hohorst, Kreutz & Bücher, 1959); acetoacetate and 3-hydroxybutyrate (Williamson, Mellanby & Krebs, 1962); glucose (Slein, 1963). Free fatty acids were determined in plasma (Itaya & Ui, 1965), as was insulin (Morgan & Lazarow, 1963, as modified by Soeldner & Sloane, 1965). Rat insulin was used as a reference standard.

Diabetic rats. Rats were made diabetic by injection of 150mg of streptozotocin/kg into the tail vein, a dose sufficient to eliminate pancreatic β-cell function (Junod, Lambert, Stauffacher & Renold, 1969). One day after injection, all rats were severely diabetic with urine glucose concentrations of greater than 0.5% (Clinistix test, Miles Laboratories Ltd., Elkart, Ind., U.S.A.). They were then given 2–3 units of protamine zinc insulin at 4.30p.m. each day for a period of 7 days. The dose was adjusted to each rat's requirement on the basis of weight maintenance and the presence of glucose and ketone bodies in the urine as tested with Clinistix and Ketostix. The rats lost an average of 12g the first day but maintained constant weight thereafter.

Infusion of acetoacetate and collection of blood. Two days before the experiment the rats were placed in restraining cages to allow familiarization with the cage conditions. On the penultimate day the rats were anaesthetized with diethyl ether and catheters were placed in the femoral artery and vein as previously described (Hawkins *et al.* 1971). Acetoacetate was infused at either 150μmol/min per kg for 10min and 75μmol/min per kg thereafter to produce blood concentrations of ketone bodies in fed rats comparable with those occurring after 48h starvation (2–3mM) or at rates double those stated to produce abnormally high ketone-body concentrations (6–7mM). Blood (0.25–0.45ml) was sampled at intervals from the arterial catheter and coagulation was retarded by adding 0.2M neutral EDTA to a final concentration of 3–5mM. Between sampling the cannulae were kept open with 0.154M-NaCl. The total amount of blood removed from a rat never exceeded 1.8ml. A portion (0.1ml) of the blood was acidified in 2ml of 0.3M-$HClO_4$ for metabolite measurements. The plasma from the remainder was separated immediately by centrifugation for measurement of free fatty acids and insulin.

Anaesthetized rats. All experiments were done on conscious rats with the exception of one group which were anaesthetized with 30mg of freshly made Nembutal aqueous solution/kg administered intravenously 45min before infusion.

RESULTS

Blood metabolite concentrations in restrained, catheterized rats. The method by which blood samples are taken and the treatment of rats may affect metabolite concentrations. Table 1 contains a comparison of metabolite concentrations of mixed blood from unanaesthetized decapitated rats, arterial blood of freshly anaesthetized rats (50mg of Nembutal/kg administered 30–45min before sampling) and arterial blood of conscious restrained rats prepared as described in the Materials and Methods section.

The concentrations of glucose and free fatty acids did not differ between the various conditions in either the fed or the starved group.

Blood from decapitated rats was primarily venous and the lactate concentrations were higher than arterial blood in both fed and starved rats. This is probably due to anoxia and the consequent high rate of lactate production that inevitably occurs after decapitation. The arterial lactate concentration of fed restrained rats was about half that of fed anaesthetized rats. Starved restrained rats had arterial lactate concentrations about 67% higher than blood of anaesthetized rats.

The concentration of ketone bodies in fed restrained rats was three- to four-fold higher and starved restrained rats had concentrations 1.27–1.72mM higher than either anaesthetized rats or

Table 1. *Blood metabolites obtained under various conditions in fed and* 48h-*starved rats*

The results are mean values (±S.D.) with the number of observations in each group in parentheses. Fed and starved decapitated rats were unanaesthetized and blood was obtained on exsanguination. Anaesthetized rats were given an intraperitoneal injection of Nembutal (50mg/kg) 30–45min before blood was obtained from the femoral artery. Conscious rats were restrained for 2 days before sampling and had an arterial catheter placed in the femoral artery on the penultimate day.

Conditions	Glucose (mM)	Lactate (mM)	Pyruvate (mM)	3-Hydroxybutyrate (mM)	Acetoacetate (mM)	Total ketone bodies (mM)	Free fatty acids (mequiv./l)
Fed, decapitated, mixed blood (6)	6.14±0.14	1.76±1.43	0.142±0.070	0.03±0.03	0.12±0.01	0.15	0.64±0.23
Fed, anaesthetized, arterial blood (9)	6.34±0.47	1.10±0.57	0.141±0.053	0.09±0.04	0.13±0.03	0.22	—
Fed, conscious, arterial blood (23)	6.26±0.62	0.59±0.23	0.098±0.057	0.26±0.18	0.39±0.16	0.65	0.66±0.35
48h-starved, decapitated, mixed blood (6)	4.06±0.21	2.15±0.71	0.234±0.075	1.83±0.53	0.53±0.09	2.36	1.42±0.34
48h-starved, anaesthetized, arterial blood (10)	4.76±0.92	0.54±0.15	0.059±0.008	2.00±0.49	0.81±0.16	2.81	—
48h-starved, conscious, arterial blood (13)	3.87±0.56	0.90±0.28	0.120±0.035	2.63±0.91	1.45±0.29	4.08	1.31±0.38

decapitated rats. The reason for this is not clear.

Infusion of acetoacetate into normal fed rats. Infusion of acetoacetate to raise the total ketone-body concentration to values approximating to those found in starvation (2–3mM) did not change the arterial glucose concentration significantly (Table 2). The plasma insulin concentration rose by 53% within 10min and decreased slightly throughout the remainder of the infusion period. Rapid acetoacetate infusion resulting in higher concentrations (6–7mM) depressed the glucose concentration within 10min by 0.61mM and by 1.05mM at 60min. This was accompanied by a 150% rise in plasma insulin during the first 10min and a decrease in free fatty acids.

Nembutal-anaesthetized rats. Anaesthetized rats infused at the rapid rate showed a similar pattern but the increase in plasma insulin concentration was greater than in unanaesthetized rats throughout the infusion period ($P<0.1$, 0.025 and 0.05 at 10, 30 and 60min respectively).

Infusion of fed–diabetic rats. The fed, insulin-treated diabetic rats showed no consistent change in blood glucose on acetoacetate infusion at the high rate. The concentration of plasma insulin remained constant, as was to be expected. However, free fatty acids decreased by 23% within 10min and remained constant thereafter.

Infusion of normal starved rats. Infusion of acetoacetate into 48h-starved rats at the high rate resulted in an increase of total ketone bodies from 3.88mM to 10.8mM in 10min (Table 3). At 30min after the infusion was terminated the ketone-body concentrations were about twice their original value (7.58mM). There was no significant change in blood glucose throughout the experiment, but there was a threefold rise in the plasma insulin concentration, which was maintained until the infusion was stopped at 30min, whereupon the insulin concentration returned to normal by 60min. Free fatty acid concentrations decreased by 32% within 10min and remained low throughout.

DISCUSSION

The results presented here show that plasma insulin concentrations increase when the blood ketone-body concentrations are raised from less than 1mM to 2–3mM or 6–7mM in fed rats and from 3mM to 11mM in 48h-starved rats. This effect was much larger in fed rats than in starved ones, although in the fed state the initial concentrations of insulin were about 4–5 times higher than in the starved state. Madison *et al.* (1964), who first observed this effect on dogs, suggested that the stimulation of insulin secretion by ketone bodies

Table 2. *Effect of acetoacetate infusion on circulating metabolite and insulin concentrations in fed rats*

The results are mean values (±S.E.M.) with the number of observations in each group in parentheses. The symbols* and ** indicate statistical significance, as compared with the initial value, of 5 and 1% respectively. Infusions were begun at zero time and continued throughout the experiment. For details see the Materials and Methods section.

Conditions	Time (min)	Glucose (mM)	3-Hydroxy-butyrate (mM)	Aceto-acetate (mM)	Free fatty acids (mequiv./l)	Insulin (μunits/ml)
Acetoacetate-infused at slow rate (6)	0	6.78±0.24	0.49±0.15	0.47±0.09	—	26.7± 3.6
	10	6.72±0.43	1.10±0.10	2.63±0.22	—	41.0±12.4*
	20	6.44±0.09	1.02±0.10	2.27±0.14	—	39.0±10.7*
	60	6.72±0.16	0.87±0.18	2.00±0.19	—	35.2± 8.5
Acetoacetate-infused at rapid rate (9)	0	6.16±0.19	0.32±0.07	0.50±0.04	0.82±0.14	20.0± 2.7
	10	5.55±0.21*	0.92±0.07	4.99±0.26	0.73±0.10	49.9± 7.8*
	30	5.21±0.24**	1.11±0.10	4.88±0.25	0.65±0.10*	28.6± 4.2
	60	5.11±0.24**	1.25±0.11	5.12±0.30	0.64±0.07*	31.7± 6.5
Acetoacetate-infused at rapid rate, Nembutal-anaesthetized (5)	0	5.77±0.23	0.22±0.06	0.49±0.05	—	30.0± 7.1
	10	5.49±0.20	1.08±0.11	5.85±0.42	—	101.2±26.3*
	30	4.54±0.14**	1.29±0.08	6.01±0.52	—	57.5±11.8*
	60	4.64±0.28*	1.41±0.16	6.19±0.90	—	67.6±17.3*
Insulin-maintained diabetic rats, acetoacetate-infused at rapid rate (9)	0	3.32±0.84	0.19±0.06	0.85±0.13	0.83±0.13	46.9± 9.6
	10	3.76±0.99	0.95±0.13	5.43±0.71	0.64±0.01*	43.0± 8.9
	30	3.58±1.26	1.12±0.12	5.81±0.60	0.58±0.08**	32.6± 8.8
	60	4.40±1.92	1.57±0.26	6.74±0.69	0.65±0.06	41.4±11.0
NaCl-infused (8)	0	5.98±0.19	0.26±0.07	0.35±0.04	0.50±0.07	28.7± 5.5
	10	6.11±0.29	0.27±0.06	0.32±0.05	0.47±0.08	31.3± 4.2
	30	6.12±0.22	—	0.35±0.06	0.48±0.11	22.5± 5.0
	60	6.11±0.24	0.10±0.02	0.36±0.05	0.58±0.09	30.0± 4.3

Table 3. *Effect of acetoacetate on blood metabolites and insulin concentration in* 48 h-*starved rats*

The results are mean values (± S.E.M.) with the number of observations in each group in parentheses. The symbols * and ** indicate statistical significance as compared with the initial value, of 5 and 1% respectively. Infusions were begun at zero time and terminated after 30 min. For details see the Materials and Methods section.

Conditions	Time (min)	Glucose (mM)	3-Hydroxybutyrate (mM)	Acetoacetate (mM)	Free fatty acids (mequiv./l)	Insulin (μunits/l)
Acetoacetate-infused at rapid rate (8)	0	4.00±0.19	2.44±0.33	1.44±0.11	1.16±0.14*	6.4±1.7
	10	4.20±0.24	3.07±0.35	7.77±0.49	0.79±0.13*	18.5±3.5**
	30	3.61±0.29	3.23±0.35	8.53±0.57	0.80±0.10**	18.5±5.4*
	60	3.76±0.31	3.64±0.45	3.94±0.46	0.77±0.10*	7.5±1.7
NaCl-infused (5)	0	3.66±0.31	2.95±0.42	1.47±0.16	1.38±0.10	4.2±1.0
	10	4.10±0.40	2.84±0.31	1.55±0.09	1.32±0.17	7.4±1.3
	30	3.83±0.42	2.90±0.41	1.44±0.14	1.13±0.21	5.8±1.0
	60	3.75±0.30	3.09±0.47	1.50±0.19	1.23±0.20	4.5±0.8

may be of importance in the feed-back regulation of ketone-body production. Increased insulin concentrations decrease the free fatty acid release from adipose tissue (see Scow & Chernick, 1970) and therefore inhibit ketogenesis by lowering the concentrations of their precursors. In addition there is evidence that insulin may have a direct effect on hepatic ketogenesis (Bieberdorf, Chernick & Scow, 1970).

A feed-back control not mediated by insulin appears to be a direct inhibition of free fatty acid release from adipose tissue by ketone bodies (Björntorp, 1966; Hellman, Senior & Goodman, 1969). This effect occurs in alloxan-diabetic dogs where insulin is not likely to be involved (Björntorp & Scherstén, 1967). The finding that infusion of acetoacetate decreased free fatty acid concentrations without any insulin change in streptozotocin-diabetic rats (Table 2) confirms previous work. It is therefore likely that in normal animals the decrease of free fatty acids is due to the operation of both feed-back mechanisms.

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