Effects of ketone bodies on insulin release and islet-cell metabolism in the rat

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1. Ketone bodies promote insulin secretion from isolated rat pancreatic islets in the presence of 5 mM-glucose, but are ineffective in its absence. 2. At concentrations of 10 mM or less, the relative abilities of the ketone bodies to potentiate release are in the order D-3-hydroxybutyrate > DL-3-hydroxybutyrate > acetoacetate. 3. The response curve relating insulin release to D-3-hydroxybutyrate concentration displays a threshold at 1 mM and a maximum at 10 mM. 4. D-3-Hydroxybutyrate (5 mM, but not 10 mM) promotes insulin secretion in the presence of 5 mM concentrations of both L-arginine and DL-glyceraldehyde, but not with L-leucine, L-alanine, L-glutamate or 4-methyl-2-oxopentanoate. 5. The oxidation rates of the exogenous ketone bodies do not correlate well with their capacities to promote insulin release. Moreover, the oxidation of 5 mM-D-3-hydroxybutyrate can be inhibited by 25% with methylmalonate (10 mM) without any diminution of release. 6. The potentiation with D-3-hydroxybutyrate occurs without an observable increase in total islet cyclic AMP. However, a small net efflux matches the relative abilities of the ketone bodies to promote insulin release. 7. With islets from 48 h-starved animals the insulin response is both diminished and less sensitive than in fed animals, since insulin secretion is not significantly raised until a threshold of 5 mM-D-3-hydroxybutyrate is reached. 8. These results suggest that, in the rat at least, there should be a reappraisal of the physiological role of ketone bodies in the promotion of insulin release.

Among the major types of metabolite used to examine the insulin-secretory process, ketone bodies have been probably the most neglected. This is especially surprising, since ketone-body-induced insulin release has been assigned a role in the regulation of whole-body metabolism (Newsholme, 1976). However, the effects of ketone bodies on insulin release have been controversial. Early studies *in vivo* suggested that ketone-body infusions are accompanied by increases in plasma insulin in dogs (Madison *et al.*, 1964) and in man (Pi-Sunyer *et al.*, 1969; Balasse *et al.*, 1970; Owen *et al.*, 1973). However, in other studies ketone bodies did not affect blood insulin in humans (Balasse & Ooms, 1968; Binkiewicz *et al.*, 1974; Balasse & Neef, 1975). Further studies with the perfused rat pancreas indicated that these effects were glucose-dependent (Goberna *et al.*, 1974; Matschinsky *et al.*, 1975). The effect of low concentrations of ketone bodies on insulin release was never assessed in those experiments, nor was the naturally occurring D-isomer of 3-hydroxybutyrate tested. Experiments with islets isolated from either rats (Montague & Taylor, 1968) or humans (Grant *et al.*, 1980) have

again been limited. In the present study, islets isolated from fed and 48 h-starved rats have been used to characterize the insulin response to ketone bodies, and the physiological significance of these responses has been discussed. In addition, we have examined the effects of ketone bodies on some aspects of islet-cell metabolism generally associated with the secretory process. A preliminary report on some aspects of this work has appeared (Biden & Taylor, 1982).

Experimental

Animals

Male Sprague-Dawley rats (150–200 g) were allowed free access to water and to standard pellet food. They were killed by cervical dislocation.

Materials

Unless otherwise stated, all biochemical reagents were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. Human insulin standard was from Wellcome Research Reagents, Beckenham, Kent, U.K., and cyclic AMP radioimmunoassay kits

were from New England Nuclear, Southampton, U.K. All other radiochemicals were supplied by Amersham International, Amersham, Bucks., U.K.

Sodium acetoacetate was prepared by equimolar hydrolysis of ethyl acetoacetate (Krebs & Eggleston, 1945), followed by freeze-drying and standardization by enzymic analysis (Mellanby & Williamson, 1974). Sodium D-3-hydroxybutyrate was produced from the enzymic reduction of acetoacetate with 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) and a NADH generating system (Passingham & Barton, 1975). The incubation mixture was then continuously refluxed with diethyl ether, and the extracted acid neutralized and freeze-dried. Its purity was verified enzymically (Williamson & Mellanby, 1974). This method was used to measure D-3-hydroxybutyrate in blood.

Isolation and incubation of islets

Islets were isolated by pancreatic digestion with collagenase (Howell & Taylor, 1968). Throughout this procedure bicarbonate-buffered medium was used (Gey & Gey, 1936), equilibrated with O₂/CO₂ (19:1) and containing bovine serum albumin (1 mg/ml) and glucose (5 mM). For the experimental incubations other additions to the medium were sometimes substituted for glucose. When these additions were in the form of a sodium salt, the total sodium concentration in the medium was suitably re-adjusted. All incubations were performed at 37°C in a shaking water bath.

Studies on insulin release

Batches of five islets were incubated for 1 h in 1 ml of experimental medium. Samples (0.5 ml) were taken and diluted, and the insulin content was determined by radioimmunoassay (Hales & Randle, 1963), with human insulin as standard. In our laboratory, the curves for human and rodent insulin are superimposable over the range of insulin values used in these experiments.

Studies on cyclic AMP metabolism

For determination of total cyclic AMP concentration, batches of 15 islets were preincubated with the isolation medium for 10 min. After spinning down (1000g for 30s) and the removal of the supernatant, the islets were incubated with 100 µl of experimental medium. After 10 min 200 µl of ice-cold 0.1 M-HCl (containing 1 mM-3-isobutyl-1-methyl-xanthine) was added, and the incubation tubes were immediately placed in liquid N₂. On thawing, the samples were sonicated in a Branson B-15 cell disruptor (power output setting 2 × 10s). Samples (100 µl) of the sonicated preparation were diluted and cyclic AMP content was determined directly by radioimmunoassay (Steiner *et al.*, 1972).

Measurements of cyclic AMP efflux have been

previously described in detail (Campbell & Taylor, 1981). In a minor departure from this method, 25 islets were incubated in 125 µl for 30 min. Cyclic AMP was assayed as described above.

Studies on ketone-body and glucose oxidation

Before use, D-3-hydroxy[3-¹⁴C]butyrate (3.4 Ci/mol) and DL-3-hydroxy[3-¹⁴C]butyrate (3.0 Ci/mol) were treated with 0.01 M-HCl and then freeze-dried to eliminate 'carbonate-like' impurities (Berne, 1975). Groups of 15 islets were transferred to Microfuge tubes (Beckman, 1.5 ml) from which the top 10 mm had been removed. The islets were spun down (1000g for 30s) and the supernatants removed. Then 100 µl of incubation medium, containing bovine serum albumin (0.1 mg/ml) plus additions and radiolabelled substrates, was added and the tubes were incubated for 60 min.

¹⁴CO₂ was trapped and determined by using the apparatus of Sissons (1976). Metabolism was arrested by the injection of 50 µl of antimycin A (0.05 mM) dissolved in 70% (v/v) ethanol, followed by 50 µl of potassium phosphate buffer (0.4 M; pH 6) to liberate the CO₂ (Edwards *et al.*, 1972). After a further 3 h incubation, over 95% of the released CO₂ was trapped, as shown in control tubes containing NaH¹⁴CO₃, which were incubated concurrently. Non-specific radioactivity (c.p.m.) was corrected for with controls containing no islets, and for all concentrations of all substrates was less than 55% of the total radioactivity. The samples were counted for radioactivity in an Intertechnique SL300 liquid-scintillation counter, and corrected by using an external standard.

Presentation of results

All results are presented as means ± S.E.M., with the numbers of observations in parentheses. The statistical significance of differences between experimental groups was determined by Student's *t* test.

Results

Effects of ketone bodies on insulin release from isolated islets

In the absence of glucose, neither 5 mM nor 15 mM concentrations of the ketone bodies were able to promote insulin release (Table 1). Indeed, the responses evoked by these compounds were significantly less than that of the 5 mM-glucose control. However, ketone bodies substantially potentiated insulin release in the presence of 5 mM-glucose (Table 2). At equimolar concentrations, apart from 15 mM, the naturally occurring D-3-hydroxybutyrate was more effective in promoting insulin secretion than was either DL-3-hydroxybutyrate or acetoacetate. Moreover, 3 mM-D-3-hydroxybutyrate could provoke release, whereas the threshold for

Table 1. *Effects of ketone bodies alone on insulin release from isolated rat islets*

Groups of five islets were incubated for 60 min in 1 ml of bicarbonate-buffered medium (see the Experimental section) containing additions as described. Insulin released was measured by radioimmunoassay. Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Results that differ from the 5 mM-glucose control with $P < 0.05$ denoted by *.

Addition	Insulin release (pg/min per islet)
Glucose (5 mM)	2.84 $\pm$ 0.28 (16)
(20 mM)	52.88 $\pm$ 4.75 (16)*
D-3-Hydroxybutyrate (5 mM)	1.88 $\pm$ 0.20 (8)*
(15 mM)	1.76 $\pm$ 0.16 (8)*
DL-3-Hydroxybutyrate (5 mM)	2.24 $\pm$ 0.20 (5)*
Acetoacetate (5 mM)	2.04 $\pm$ 0.20 (5)*
(15 mM)	1.76 $\pm$ 0.32 (5)*

Table 2. *Effects of ketone bodies on insulin release from isolated rat islets in the presence of 5 mM-glucose*

Groups of five islets were incubated for 60 min in 1 ml of bicarbonate-buffered medium containing the additions as described (see the Experimental section). Insulin released was measured by radioimmunoassay. Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Values are significantly different ($P < 0.05$) from those obtained with 5 mM-glucose alone, except for 3 mM-acetoacetate plus glucose (Expt. 10). For any stated ketone-body concentration, values differ significantly ($P < 0.05$) between the substances tested, except for results of Expt. 13, when compared with those of Expt. 6. Different concentrations of the same substance also produce significantly different insulin responses, except in Expts. 6 and 3.

Expt. no.	Glucose (5 mM) plus addition	Insulin release (pg/min per islet)
1	—	3.84 $\pm$ 0.28 (34)
2	Glucose (15 mM)	38.44 $\pm$ 2.76 (25)
3	D-3-Hydroxybutyrate (3 mM)	7.96 $\pm$ 1.24 (12)
4	(5 mM)	17.76 $\pm$ 1.92 (31)
5	(10 mM)	24.32 $\pm$ 3.08 (6)
6	(15 mM)	9.00 $\pm$ 1.44 (19)
7	DL-3-Hydroxybutyrate (5 mM)	9.92 $\pm$ 1.20 (18)
8	(10 mM)	17.40 $\pm$ 1.40 (8)
9	(15 mM)	20.20 $\pm$ 2.08 (8)
10	Acetoacetate (3 mM)	2.98 $\pm$ 0.48 (14)
11	(5 mM)	5.60 $\pm$ 0.64 (27)
12	(10 mM)	7.04 $\pm$ 0.68 (10)
13	(15 mM)	11.56 $\pm$ 1.56 (17)

potentiation by acetoacetate was 5 mM. The insulin response was proportional to the concentration of acetoacetate and DL-3-hydroxybutyrate. However,

Fig. 1. *Insulin release from islets of fed and 48 h-starved rats in response to D-3-hydroxybutyrate, and oxidation of D-3-hydroxybutyrate, both in the presence of 5 mM-glucose*

For insulin-release studies, groups of five islets were incubated for 60 min in 1 ml of bicarbonate-buffered medium containing 5 mM-glucose and D-3-hydroxybutyrate as shown. Insulin released was measured by radioimmunoassay. Results (●, fed rats; ■, 48 h-starved rats) represent the means for at least eight individual determinations, with S.E.M. also shown. For oxidation studies, batches of 15 islets were incubated for 60 min in 100 μ l of bicarbonate-buffered medium containing 5 mM-glucose plus D-3-hydroxybutyrate as shown. For details of arrest of metabolism and counting of the radioactivity of the liberated $^{14}\text{CO}_2$, see the Experimental section. Results (▲) are presented as the means for at least six individual determinations, with S.E.M. also shown.

with the D-isomer the maximal insulin release occurred at 10 mM and was diminished but not abolished at 15 mM.

These characteristics of potentiation by D-3-hydroxybutyrate are shown more clearly in Fig. 1. In this study the response curves are shown for islets isolated from both fed and 48 h-starved rats. With fed animals, insulin release was significantly enhanced with as little as 1 mM-D-3-hydroxybutyrate ($P < 0.05$). However, starvation raised this threshold to 5 mM and markedly diminished the overall response.

The ability of D-3-hydroxybutyrate to promote insulin release in conjunction with agents other than glucose is shown in Table 3. Thus D-3-hydroxybutyrate (5 mM, but not 10 mM) was effective in potentiating with L-arginine and DL-glyceraldehyde, but not with L-leucine, 4-methyl-2-oxopentanoate, L-alanine or L-glutamine (all 5 mM).

Effects of ketone bodies on islet cyclic AMP metabolism

The total cyclic AMP measured in Table 4 represents the sum of the cyclic AMP accumulated

Table 3. *Effect of D-3-hydroxybutyrate, in the presence of various test compounds, on insulin release from isolated rat islets*

Concn. of test compound	Test compound	Insulin release (pg/min per islet)					
		L-Leucine	L-Arginine	4-Methyl-2-oxopentanoate	DL-Glycerinaldehyde	L-Alanine	L-Glutamine
5 mM alone		3.52 ± 0.48 (9)	4.68 ± 0.64 (10)	3.52 ± 0.56 (9)	4.68 ± 0.36 (10)	1.67 ± 0.12 (8)	1.44 ± 0.20 (10)
5 mM plus D-3-hydroxybutyrate (5 mM)		2.92 ± 0.48 (10)	7.48 ± 0.52 (10)*	4.48 ± 0.64 (10)	13.8 ± 1.24 (9)*	1.39 ± 0.11 (9)	1.74 ± 0.17 (9)
5 mM plus D-3-hydroxybutyrate (10 mM)		2.88 ± 0.60 (10)	5.08 ± 0.28 (9)	3.92 ± 0.68 (10)	5.52 ± 0.72 (8)	1.71 ± 0.15 (10)	1.54 ± 0.12 (9)
20 mM alone		6.68 ± 0.92 (8)*	9.24 ± 1.08 (9)*	35.8 ± 7.52 (9)*	23.1 ± 1.76 (8)*	1.52 ± 0.14 (10)	1.40 ± 0.20 (9)

Groups of five islets were incubated for 60 min in 1 ml of bicarbonate-buffered medium (see the Experimental section) containing additions as described. Insulin released was measured by radioimmunoassay. Mean values ($\pm$ s.e.m.) are shown, together with the numbers of individual determinations in parentheses. Comparisons were made between the response to each test substance alone at 5 mM and the effect of various additions. Differences where $P < 0.05$ are denoted by *.

Table 4. *Effects of ketone bodies on total cyclic AMP accumulation in, and cyclic AMP efflux from, isolated rat islets*

For details of methods see under relevant heading in Experimental section. Mean values ($\pm$ s.e.m.) are shown, together with the numbers of individual determinations in parentheses. Values significantly different ($P < 0.05$) from the respective 5 mM-glucose control are represented by *.

Glucose (5 mM) plus addition	Total cyclic AMP (fmol/islet)	Cyclic AMP efflux (pmol/30 min per 50 islets)
—	5.8 ± 0.5 (10)	0.012 ± 0.004 (5)
D-3-Hydroxybutyrate (5 mM)	4.7 ± 0.8 (9)	0.164 ± 0.008 (5)*
D-3-Hydroxybutyrate (10 mM)	7.4 ± 1.4 (6)	—
Acetoacetate (5 mM)	—	0.026 ± 0.004 (4)*
Theophylline (5 mM)	18.0 ± 3.9 (4)*	—
Glucose (15 mM)	12.0 ± 3.4 (4)*	0.330 ± 0.097 (5)*

intracellularly plus that small component effluxed during the 10 min incubation period. The cyclic AMP efflux alone, as determined in a different series of experiments, is also shown in Table 4. D-3-Hydroxybutyrate (5 or 10 mM), in the presence of glucose, was unable to promote a detectable rise in total cyclic AMP accumulation. However, control incubations with 20 mM-glucose, and with 5 mM-glucose plus 5 mM-theophylline, did increase intracellular cyclic AMP. In contrast, both ketone bodies (5 mM), when used in conjunction with 5 mM-glucose, induced significant rises in cyclic AMP efflux. In keeping with their relative abilities to promote insulin release, the effect with D-3-hydroxybutyrate was much more pronounced than that of acetoacetate. The 20 mM-glucose control was more effective still.

Ketone-body oxidation in isolated islets

At 5 mM concentrations, D-3-hydroxybutyrate and DL-3-hydroxybutyrate were oxidized at 4.1 ± 1.2 (5) and 3.8 ± 0.7 (9) pmol/h per islet respectively. There was no significant difference between these values despite the different capacity of these compounds for promoting insulin release. Moreover, these rates of utilization were unaffected by the presence of 5 mM-glucose. In addition, neither D-3-hydroxybutyrate nor DL-3-hydroxybutyrate (5 or 15 mM) exerted any effect on the oxidation of 5 mM-glucose (results not shown). Methylmalonate (10 mM) gave a 25% inhibition of oxidation of 5 mM-D-hydroxybutyrate, in the presence of 5 mM-glucose, but had no effect on the insulin release evoked

Table 5. Oxidation of D-3-hydroxybutyrate compared with its effects on insulin release in isolated islets with and without methylmalonate

For details of the measurement of insulin release and hydroxybutyrate oxidation see under appropriate headings in the Experimental section. Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. A significant difference ($P < 0.05$) between values with and without methylmalonate is represented by *.

Addition	D-3-Hydroxybutyrate (pmol/h per islet)	Insulin release (pg/min per islet)
D-Glucose (5 mM) plus D-3-hydroxybutyrate (5 mM)	2.8 $\pm$ 0.2 (8)	5.53 $\pm$ 0.50 (18)
D-Glucose (5 mM) plus D-3-hydroxybutyrate (5 mM) plus methyl- malonate (10 mM)	2.1 $\pm$ 0.1 (8)*	6.53 $\pm$ 0.65 (17)

by these compounds (Table 5). In contrast, methylmalonate (10 mM) affected neither CO_2 production from glucose (5 mM) nor glucose-induced insulin release (results not shown). The oxidation response curve for D-3-hydroxybutyrate is shown in Fig. 1. This does not correlate at all well with that for insulin release (Fig. 1, ●).

In those experiments where blood D-3-hydroxybutyrate was measured, the value was 1.40 ± 0.22 mM (mean $\pm$ S.E.M.) after starving the rats for 48 h.

Discussion

Characteristics of the insulin response to ketone bodies

In this paper a 5 mM-glucose response has been used as a control with which to compare the secretory capacity of the experimental additions. This concentration of glucose is sub-stimulatory for insulin release, but helps to avert the decline in ATP concentration seen when islets are incubated in the absence of exogenous substrate (Ashcroft *et al.*, 1973). Indeed, DL-3-hydroxybutyrate is less effective than glucose in maintaining islet ATP concentrations (Ashcroft *et al.*, 1973), and this may explain why the insulin responses to ketone bodies are relatively low (Table 1). Insulin release was, however, potentiated by ketone bodies in the presence of sub-stimulatory concentrations of glucose (Table 2). Although not previously demonstrated with isolated islets, nor with the naturally occurring D-3-hydroxybutyrate, these results do

confirm earlier studies in other systems (Jenkins *et al.*, 1970; Metzgar *et al.*, 1973; Goberna *et al.*, 1974; Matschinsky *et al.*, 1975). The D-isomer was twice as effective a potentiator as the DL mixture, since the responses evoked by 3 mM-D- and 5 mM-DL-, and by 5 mM-D- and 10 mM-DL-, 3-hydroxybutyrate were approximately equal. Since D-3-hydroxybutyrate constituted $41 \pm 1\%$ of the commercial DL preparation, it appears that the D-component of the mixture was responsible for the insulin release, whereas the L isomer was largely ineffective.

The inability of D-3-hydroxybutyrate to potentiate insulin release with L-leucine and with 4-methyl-2-oxopentanoate (Table 3) may be due to the inhibition of branched-chain amino acid metabolism by ketone bodies seen in other tissues (Palaiologos & Felig, 1976). Nor does D-3-hydroxybutyrate promote insulin release in conjunction with L-alanine or L-glutamine (Table 3). Although they are not initiators of insulin release, these compounds are then two main amino acids circulating during starvation. Moreover, the lack of effect with glutamine would seem to rule out an interaction of ketone bodies with glutamate dehydrogenase (EC 1.4.1.2), a possible regulatory site in the action of some non-metabolizable secretagogues (Panten *et al.*, 1980; Sener & Malaisse, 1980).

Ketone bodies and islet-cell cyclic AMP metabolism

Insulin release is generally associated with an increase in islet-cell cyclic AMP (Campbell & Taylor, 1981). However, the two processes have been dissociated, and it is now generally held that the increase in cyclic AMP is not a primary trigger of insulin release, but acts rather as a modulating response (Hedekov, 1980). This concept is indirectly supported by the observations that D-3-hydroxybutyrate was unable to produce a detectable increase in total islet cyclic AMP (Table 4), and yet theophylline (5 mM) was able to potentiate further D-3-hydroxybutyrate (5 and 15 mM)-induced insulin release in the presence of glucose (5 mM). Thus an increase in cyclic AMP may not be necessary to trigger ketone-body-induced insulin release, but if that rise is subsequently produced by other means, there is a further enhancement of the insulin response. However, the results reported here do not preclude a transitory but undetectable rise in total cyclic AMP content.

Cyclic AMP efflux is a phenomenon that correlates better with the secretory capacity of many agents than does cyclic AMP accumulation (Campbell & Taylor, 1981). This is also true for ketone bodies, since D-3-hydroxybutyrate promoted a much larger efflux than did acetoacetate (Table 4). However, the relevance of this phenomenon to the secretory process remains unknown.

Oxidation of ketone bodies and glucose

In a previous study with isolated islets, the oxidation rates of ketone bodies were less than 10% of those found in the kidney cortex (Berne, 1975). In our study, 15 mM-D-3-hydroxybutyrate was oxidized at only half the rate of 5 mM-glucose (results not shown). Thus it appears that ketone bodies are not major energy substrates in the islets of fed rats.

Although it has been proposed that ketone bodies inhibit glucose oxidation (Randle *et al.*, 1964), it was subsequently proposed that they do so only in tissues with a high glycolytic rate, such as the heart, diaphragm and brain (Maizels *et al.*, 1977). Pancreatic islets are not as active as these tissues in the utilization of glucose, and this could explain the lack of effect of ketone bodies on islet glucose oxidation.

The potentiation of glucose-induced insulin release, seen with such agents as fructose, correlates well with the combined rates of oxidation of the initiator plus potentiator (Zawalich *et al.*, 1977). Such a simple explanation does not seem to hold for ketone bodies, because the combined metabolic rates of glucose plus D-3-hydroxybutyrate, and of glucose plus DL-3-hydroxybutyrate, appear similar, despite their different capacities to promote insulin release. Possibly the two isomers are metabolized by different pathways, of which that used by the D component is the more important for insulin release. D-3-Hydroxybutyrate is probably utilized in the regular extrahepatic pathway, via D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), a known islet enzyme (Berne, 1976). The L isomer might be metabolized in the fatty acid oxidation cycle (Robinson & Williamson, 1980), since an active L-3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) has been demonstrated in islets (Ågren *et al.*, 1977). Indeed, because of the reported preference of A₂ cells for the oxidation of fatty acids (Edwards *et al.*, 1972), it is conceivable that the L isomer is metabolized chiefly in those cells, leaving D-3-hydroxybutyrate to be metabolized in, and exert its influences on, the insulin-producing B cells.

In any event, the response curves for D-3-hydroxybutyrate oxidation and for insulin release do not correspond well (see Fig. 1). This is especially true for 15 mM concentrations, at which a diminution of insulin release is accompanied by further enhancement of the oxidative rate.

A third instance of the poor association between insulin release and ketone-body oxidation is seen in the experiments with methylmalonate (Table 5). This compound is a competitive inhibitor of D-3-hydroxybutyrate dehydrogenase and has been shown to inhibit the oxidation of D-3-hydroxybutyrate in other tissues, but had no effect on acetoacetate or glucose oxidation (Patel *et al.*, 1976). In islets, methylmalonate showed a similar specificity for D-3-hydroxybutyrate oxidation, causing a 25% inhibi-

tion (Table 5). However, it had no effect either on the potentiation of insulin release by D-3-hydroxybutyrate (Table 5) or on its initiation by glucose.

Since ketone-body potentiation correlates well with neither the CO₂ production from these compounds nor with their effects on intracellular cyclic AMP metabolism, it is impossible to explain here the diminution of insulin secretion seen with higher concentrations of D-3-hydroxybutyrate (Tables 2 and 3, Fig. 1). Perhaps, as is the case with amino acids and α -oxo acids, insulin release caused by hydroxybutyrate will relate better to changes in NAD(P)H than to the parameters mentioned above (Hutton *et al.*, 1979; Malaisse *et al.*, 1982).

Physiological implications of ketone-body-induced insulin release

It has been proposed that ketone-body-induced insulin release serves to prevent a potentially fatal ketoacidosis during starvation (Madison *et al.*, 1964; Owen *et al.*, 1973; Bates & Linn, 1976). Thus raised insulin concentrations would inhibit lipolysis and thereby slow the production of ketone bodies. However, the decrease in non-esterified fatty acids seen with ketone-body infusions has been dissociated from insulin release in kinetic studies (Balasse & Ooms, 1968; Balasse & Neef, 1975) and in experiments with diabetic subjects (Hawkins *et al.*, 1971; Binkiewicz *et al.*, 1974). This suggested that ketone bodies could also exert a direct inhibitory effect on lipolysis, a hypothesis further supported by studies *in vitro* (Bjorntorp, 1966; Fain & Shepherd, 1976).

Table 2 shows that starvation has decreased the overall islet response even in the absence of D-3-hydroxybutyrate. Moreover, at concentrations below 5 mM, this compound was unable to raise the depressed insulin release. D-3-Hydroxybutyrate would rarely reach 5 mM, even in rats undergoing prolonged starvation, and indeed in our study its concentration was substantially less than this after 48 h starvation. It therefore seems unlikely that ketone bodies could regulate lipolysis through changes in the insulin concentration. Thus during starvation in the rat the direct inhibition of lipolysis by ketone bodies would seem to be the more important regulatory mechanism. In contrast, the demonstration in the control experiment that as little as 1 mM-D-3-hydroxybutyrate could potentiate insulin release indicates that ketone-body-induced insulin secretion may be important in the fed state. Such low concentrations of D-3-hydroxybutyrate may be reached in various non-starvation conditions such as post-exercise ketosis and mild diabetes (Robinson & Williamson, 1980). In the first example, raised insulin concentrations might be important to shut down quickly the fatty acid mobilization and the hepatic glucose output no longer

needed to sustain exercise. In mild diabetes associated with obesity, the relative increase in ketone bodies may contribute to the high insulin concentrations usually seen with this form of the disease. Another relevant physiological change might be during pregnancy. Although D-3-hydroxybutyrate does not normally reach 1 mM during this condition, islets obtained from pregnant rats are often hyper-responsive to secretory stimuli (Green & Taylor, 1973). Moreover, during pregnancy, subjects show an increased tendency towards mild hyperketonaemia associated with mild exercise and short periods of starvation.

Thus, although ketone-body potentiation of insulin release may be important in some non-starvation conditions, its significance as a metabolic regulator during starvation is open to doubt.

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