INHIBITION OF LIPOLYSIS IN BOVINE ADIPOSE TISSUE BY BUTYRATE AND β-HYDROXYBUTYRATE

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Received 29 July 1974

1. Introduction

In a previous paper [1] it was shown that butyrate and DL-β-hydroxybutyrate, at a concentration of 10 mM, inhibit lipolysis in bovine adipose tissue in vitro. This inhibition was observed for basal lipolysis as well as for lipolysis stimulated by noradrenalin. Acetate, propionate and acetoacetate were shown to have no effect on these processes [1].

In this paper it will be shown that the inhibition of lipolysis by β-hydroxybutyrate, contrary to the effect of butyrate, may be important in dairy cows in vivo. Both optical isomers of the ketone body exhibit the inhibitory effect. It is concluded that the inhibition of lipolysis of β-hydroxybutyrate occurs through an inhibitory effect on the formation of cAMP* in the fat cell.

2. Methods and materials

Biopsy samples of subcutaneous adipose tissue from the flank region of dairy cows in late pregnancy or early lactation were used for these studies. The details of the procedures for taking these samples and handling them in the in vitro experiments have been reported previously [1].

FFA* in the media were measured according to Trout's modification [2] of Dole's titration method [3] The procedures for estimating the amount of tissue-associated FFA have been described elsewhere [4]. Glycerol was determined enzymatically according to Eggstein and Kreutz [5], with a test set of Boehringer.

Theophylline and noradrenalin were added to the media as aqueous solutions, dibutyryl-cAMP as a solution in methanol.

The optical isomers of β-hydroxybutyric acid were purified according to McCann and Greville [6]. In the final, enriched preparations (containing 88% of the L-isomer and 93% of the D-isomer, respectively) the total acid content was determined by titration and the D-isomer was determined enzymatically [7].

Bovine serum albumin (fatty acid poor) was obtained from Fluka; noradrenalin (L-arterenol–HCl), butyrate and DL-β-hydroxybutyrate (sodium salts) were from Sigma; dibutyryl-cAMP was from Boehringer; theophylline and β-hydroxybutyric acid were from Merck and quinine was from Baker.

3. Results

The effect of butyrate on the noradrenalin-stimulated lipolysis in bovine subcutaneous adipose tissue is shown in fig. 1. At the lowest concentration of added butyrate (0.6 mM) lipolysis was inhibited to a small extent only. The degree of inhibition of lipolysis increased with in-

* *Abbreviations:* cAMP = cyclic adenosine-3′,5′-monophospha :e; FFA = free (unesterified) fatty acids.

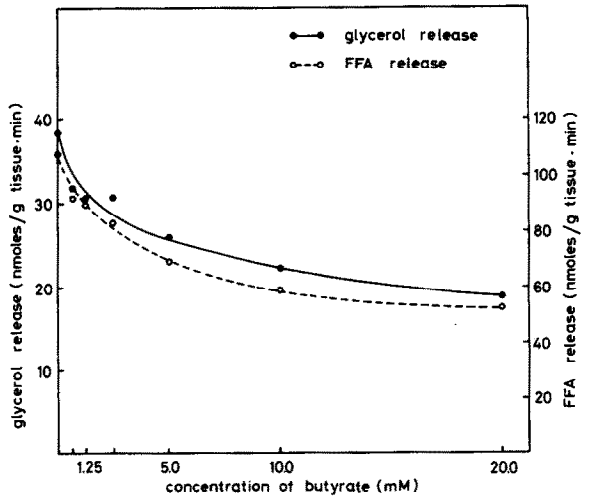

Fig. 1. Dose-response curves for the effect of sodium butyrate on the release of FFA and glycerol from bovine subcutaneous adipose tissue in the presence of 25 μM noradrenalin. Each point represents the average of triplicate incubations.

creasing butyrate concentrations up to 10 mM, and remained almost constant at higher concentrations of butyrate. At all butyrate concentrations FFA release and glycerol release were inhibited to the same extent.

Fig. 2 shows the effect of increasing concentrations

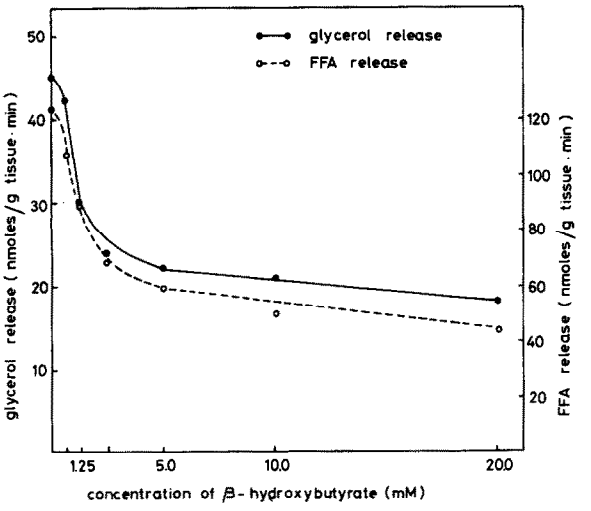

Fig. 2. Dose-response curves for the effect of sodium DL-β-hydroxybutyrate on the release of FFA and glycerol from bovine subcutaneous adipose tissue in the presence of 25 μM noradrenalin. Each point represents the average of triplicate incubations.

Table 1
Inhibition of lipolysis in bovine subcutaneous adipose tissue by various mixtures of D- and L-β-hydroxybutyrate

β-Hydroxybutyrate			Glycerol release	
D-isomer	L-isomer	Total	(nmoles/g tissue · min)	
(mM)	(mM)	(mM)	Exp. 1	Exp. 2
0	0	0	42.1	25.3
0.03	0.18	0.21	36.5	
0.33	0.02	0.35	34.6	
0.65	0.58	1.23		20.7
0.15	1.10	1.25		19.8
1.17	0.08	1.25		19.2
2.51	0.18	2.69	12.1	
0.33	2.43	2.76	13.4	
5.28	4.73	10.01		10.7
1.22	8.79	10.01		11.1
9.38	0.67	10.05		10.4

The values given are means of quadruplicate incubations. Lipolysis was maximally stimulated by addition of noradrenalin (25 μM).

of DL-β-hydroxybutyrate on the release of glycerol and FFA. Glycerol release and FFA release were inhibited equally. At a concentration of 1.25 mM, DL-β-hydroxybutyrate effected a considerable inhibition of

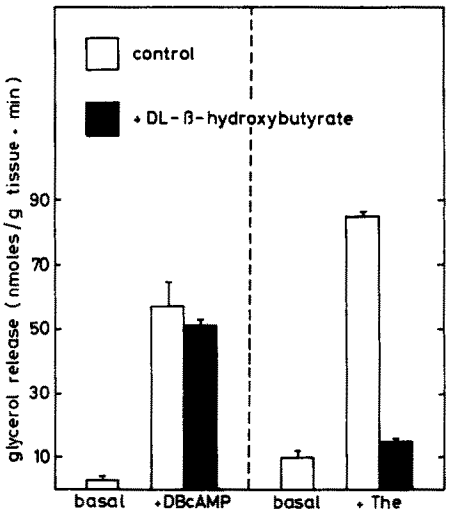

Fig. 3. Effects of 10 mM DL-β-hydroxybutyrate on lipolysis stimulated by 1 mM theophylline (The) and by 5 mM dibutyryl-cAMP (DBcAMP) in bovine subcutaneous adipose tissue. The values given are means of triplicate incubations.

Table 2
Effects of DL-β-hydroxybutyrate on the amount of tissue-associated FFA and on the rate of lipolysis in adipose tissue

Exp.	Glycerol release (nmoles/g tissue·min)		Tissue-associated FFA (μmoles/g tissue)	
	Control	With β-hydroxybutyrate (10 mM DL)	Control	With β-hydroxybutyrate (10 mM DL)
1	17.5	4.0	2.05	0.77
2	34.5	5.3	2.04	0.44
3	28.4	13.4	2.08	1.37

The values given are means of triplicate incubations. Lipolysis was maximally stimulated by addition of noradrenalin (25 μM).

lipolysis and maximal inhibition was approached at 5 mM DL-β-hydroxybutyrate.

As shown in table 1, the two optical isomers of β-hydroxybutyrate are equally active in suppressing glycerol release from bovine subcutaneous adipose tissue.

The inhibition of lipolysis by DL-β-hydroxybutyrate was accompanied by a decreased level of tissue-associated FFA in the adipose tissue (table 2).

Both dibutyryl-cAMP (5 mM) and theophylline (1 mM) stimulated lipolysis in bovine adipose tissue. As shown in fig. 3, DL-β-hydroxybutyrate did not significantly affect lipolysis stimulated by dibutyryl-cAMP but almost completely blocked the theophylline-stimulated lipolysis.

4. Discussion

Acetic, propionic and butyric acid are the major sources of metabolic energy, made available to the adult ruminant by digestion and absorption [8,9].

Of these volatile fatty acids, only butyrate affects lipolysis in bovine adipose tissue in vitro [1]. In all experiments, butyrate inhibited fatty acid release to the same extent as glycerol release, indicating that the decrease in fatty acid release resulted from an inhibition of lipolysis. Noradrenalin-stimulated lipolysis was inhibited by butyrate to a somewhat greater extent than basal lipolysis [1].

The physiological significance of these observations is rather doubtful as the inhibitory effect of 0.6 mM butyrate on the stimulated lipolysis in vitro is small (fig. 1) and the concentration of butyrate in the peripheral blood of the dairy cow fluctuates within a much lower range: 5–30 μM [10].

In contrast to acetoacetate which is without effect on lipolysis in bovine adipose tissue in vitro, DL-β-hydroxybutyrate strongly inhibits this process [1]. The inhibition by β-hydroxybutyrate may well play a role in the control of lipolysis in vivo. First, it was found that the naturally occurring D-isomer is an equally strong inhibitor of lipolysis as the L-isomer (table 1). Secondly, it has been demonstrated that during ketosis in dairy cows, the concentration of β-hydroxybutyrate in blood plasma rises from below 0.5 mM to values in the range 1–5 mM [11,12]. Fig. 2 shows that in this range the rate of lipolysis in adipose tissue is strongly influenced by the concentration of β-hydroxybutyrate in the medium. Thirdly, an in vivo effect of β-hydroxybutyrate on lipolysis may also be concluded from the reported inhibitory action of an intravenous dose of β-hydroxybutyrate on lipolysis in dogs [13, 14] and from its depression of the FFA concentration in human [15] and goat plasma [16].

Adler et al. [17] reported that in periods of rapid fat mobilization, e.g. in bovine acetonaemia, most of the ketone bodies in the blood originate from FFA. Therefore, we assume that in dairy cows a feedback mechanism is operative, in which a product derived from excessive breakdown of fatty acids participates in the regulation of fat mobilization by inhibiting lipolysis.

Lipolysis in bovine adipose tissue is stimulated by

noradrenalin [1], by dibutyryl-cAMP which is believed to activate lipolysis in the same way as endogenous cAMP, and by theophylline, a well-known inhibitor of the phosphodiesterase responsible for the breakdown of cAMP (fig. 3). These stimulatory effects indicate that the rate of lipolysis in bovine adipose tissue is dependent on the activity of a hormone-sensitive lipase, regulated according to the two-messenger concept of Sutherland et al. [18].

β-Hydroxybutyrate inhibits lipolysis stimulated by noradrenalin and by theophylline, but is ineffective against lipolysis stimulated by dibutyryl-cAMP (fig. 3). It may be concluded, therefore, that β-hydroxybutyrate acts on lipolysis by lowering the cAMP level in the fat cell rather than by interacting with the hormone-sensitive lipase-activating system beyond it. Since it has been demonstrated that β-hydroxybutyrate is unable to reactivate the theophylline-inhibited phosphodiesterase [19], it is unlikely that the lower cAMP level is brought about by an increased cAMP breakdown. This suggests an interaction of β-hydroxybutyrate with adenylate cyclase but, as in rat adipose tissue [19,20], not specifically with the adrenergic receptor sites of the cyclase system, since basal lipolysis is also inhibited by this ketone body [1].

The antilipolytic action of β-hydroxybutyrate is not caused by its occupation of FFA-binding sites on the albumin in the medium. In that case FFA inside the adipose tissue would accumulate [4]. Table 2 shows, however, that the level of tissue-associated FFA is even lowered in the presence of β-hydroxybutyrate. Neither is its inhibitory effect based on competition with FFA for the fatty acid receptor sites inside the adipose tissue, since in that case β-hydroxybutyrate would inhibit lipolysis stimulated by dibutyryl-cAMP to the same extent as lipolysis stimulated otherwise.

The results in this paper do not support our earlier speculation [1] that in ruminants butyrate has taken over the function of glucose as the main metabolic regulator of fat mobilization. The overall regulation of fat mobilization in bovine adipose tissue is discussed in more detail elsewhere [21,22]. It is clear, however, from the data reported in this paper that β-hydroxybutyrate may play an important role in the regulation of fat mobilization in ruminants, especially in conditions of acetonaemia.

Acknowledgements

The authors thank Dr I. Mulder for fruitful discussions and Mrs W. Franklin, Mr R. Leer and Mr R. L. Stobbe for skilful technical assistance.

This research was supported by a grant of the Netherlands Organization for Applied Scientific Research (TNO), the Hague.

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