Published in final edited form as:

Blood Cells Mol Dis. 2002 ; 29(1): 48–56.

Induction of Fetal Hemoglobin by Propionic and Butyric Acid Derivatives: Correlations between Chemical Structure and Potency of Hb F Induction¹

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Abstract

Short-chain fatty acids (C2-C9) induce fetal hemoglobin synthesis in primary cell cultures, primates, and patients. We carried out experiments to test whether relationships exist between chemical structure and the Hb F-inducing potential of several short-chain fatty acid derivatives. BFUe cultures were performed in the presence of propionic and butyric congeners, covering the full spectrum of substitutions of these molecules, including polar and non-polar groups, esters, and double bonds. We found that the fetal hemoglobin inducibility is related to the chemical structure of the inducing compound. This structure–activity relation depends on the length of carbon chain, the nature of the substitutions, and the position of more potent substitutions on the carbon chain. It appears that substitutions enhancing the inducibility of these compounds are (with decreasing potency): methyl > phenyl > hydroxy >> amino groups. Placement of these substitutions at a position distal to the carboxyl group enhances γ -globin inducibility. Presence of the carboxyl group is prerequisite for γ -globin inducibility.

Introduction

Pharmacological induction of fetal hemoglobin (Hb F) in humans and other primates has been achieved with several agents. Hb F-inducing agents include (a) cytotoxic drugs, which induce Hb F by altering the kinetics of erythropoiesis (summarized in 1); (b) hemopoietins, such as erythropoietin, which act by inducing rapid erythroid regeneration (2); and (c) short-chain fatty acids and their analogues (3–13). Butyrate (10, 12) and phenylbutyrate (11) have been used in the treatment of β thalassemia and sickle cell disease with variable results. More recently it has been shown that intermittent administration of butyric acid produces consistent induction of Hb F in patients with sickle cell disease (13). Other short-chain fatty acids have not been used for treatment of humans, except for valproic acid, an anticonvulsant, which has been found to produce small increases in F cells in patients with epilepsy (8, 14). Development of new short-chain fatty acid-based therapeutics is therefore desirable. For this reason, we decided to examine possible relationships between the chemical structure of short-chain fatty acids and their Hb F-inducing potential. Primary cultures were performed in the presence of short-chain fatty acid congeners, chosen to cover most aspects of the potential structure–activity relationship. Our experiments focused on propionic and butyric acid congeners, covering the full spectrum of substitutions of these molecules. For the completeness of the study we included

¹This work was supported by: National Institutes of Health Grant R37 HL20899.

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two kinds of each hydrophilic (polar) and hydrophobic (non-polar), as well as carboxyl group substitutions. Our results show that the potency of fetal hemoglobin induction by short-chain fatty acid derivatives is related to the (a) length of carbon chain; (b) nature of the substitutions; and (c) position of substitutions on the carbon chain.

Materials and Methods

Compounds and Chemicals

We used derivatives of propionic and butyric acids having substitutions of one hydrogen in all possible positions of the original molecule and with different groups. The compounds were commercially available and purchased from Sigma (St. Louis, MO), Lancaster Synthesis (Windham, NH), and TCI America (Portland, OR). The linear structures of the compounds used are shown in Figs. 1 and 2.

Erythroid Cell Cultures

Cultures were performed as previously described (15). Briefly, peripheral blood was collected from normal individuals in heparinized tubes. Mononuclear cells were isolated using a Hypaque–Ficoll density gradient (Lymphoprep; Nyegaard, Oslo, Norway), resuspended in Iscove's modified Dulbecco's medium (HyClone, Logan, UT) at a concentration of 4×10^5 cells/ml, and plated in methylcellulose-supporting media containing the following components: 1% methylcellulose (Fisher Scientific, Pittsburgh, PA), 0.2 U/ml r-hEPO (Genetics Institute, Cambridge, MA), 30% charcoal-treated fetal calf serum (HyClone), 0.1 mmol/L 2-mercaptoethanol (Sigma), and varying concentrations of the respective test compound. Cell cultures were maintained in an incubator (high humidity, 37°C, 5% CO₂). After 14 days in culture, hemoglobinized colonies, derived from burst-forming-units erythroid, were identified and collected using a dissecting microscope; they were plucked, washed, and subjected to analysis.

Measurement of Hb F-Containing Cells by Immunofluorescence

For measurement of F-positive erythroblasts, BFUe colonies were washed, dispersed, fixed in methanol, and labeled by a mouse anti- γ chain monoclonal antibody followed by a goat anti-mouse F (ab')₂-fluorescein isothiocyanate antibody, as previously described (16). The percentage of γ -positive cells was determined by using a universal fluorescent microscope (Zeiss, Thornwood, NY). Staining was also performed using an anti- β chain antibody to confirm that essentially all erythroblasts contained globin. For measurement of F-positive erythroblasts triplicate counts of at least 500 cells were performed.

mRNA Analysis

Total mRNA was prepared from BFUe colonies, as described (17). Globin mRNA was analyzed by RNase protection using human γ and β probes (18, 19), yielding protected fragment sizes of 170 and 205 bp, respectively. Gamma- and β -globin mRNA was quantitated using Phosphor-Imager (Molecular Dynamics, Sunnyvale, CA).

Results

To examine the structural requirements for fetal hemoglobin induction, we studied the structure–activity relation of several derivatives of propionate and butyrate. The selected compounds can be divided into three subgroups: hydrophilic (amino and hydroxyl), hydrophobic (methyl and phenyl), and carboxylic acid derivatives (esters and amides). A range of concentrations was used in the BFUe cultures done with these compounds. Charcoal-treated FCS was used as negative control, while cultures done in the presence of the parent compound

(i.e., butyrate or propionate) were used as positive controls. The effects of each compound on fetal hemoglobin expression were assessed by measuring $\gamma/\gamma + \beta$ mRNA and γ -positive erythroblasts in cells harvested after 14 days in culture. BFUe colonies were also counted to assess the effects of these compounds on erythroid progenitor cell growth.

Amino and Hydroxy Derivatives of Propionic Acid (Fig. 1 and Table 1)

These compounds are derived from propionic acid by substituting one atom of hydrogen in positions 2 and 3 of propionic acid with amino and hydroxyl groups, respectively. The derivatives possess different physicochemical properties than the parent compound. Five commercially available derivatives were tested in primary cultures. BFUe cultures were performed in the presence of 2-amino (L and D isomers)-, 2-hydroxy-, 3-amino-, and 3-hydroxy-propionic acid. The results are presented in Table 1.

2-L-amino-propionic acid did not affect the levels of $\gamma/\gamma + \beta$ mRNA at concentrations up to 50 mM in BFUe culture media (Table 1). Similar results were obtained for its enantiomer 2-D-aminopropionic acid, which was not able to significantly increase γ mRNA levels (Table 1) at the same concentration range. Frequency in γ -positive erythroblasts paralleled those of γ mRNA (data not shown).

There was a small increase in γ mRNA level in the cultures done in the presence of 40 and 50 mM 2-hydroxy-propionic acid. Two compounds with substitutions at position 3 of propionic acid were tested in culture. 3-amino-propionic acid was added in BFUe culture media in concentrations ranging from 0 to 60 mM; there was no increase in γ mRNA. 3-hydroxy-propionic acid increased γ mRNA in the single experiment done with this compound.

Amino and Hydroxy Derivatives of Butyric Acid (Fig. 2 and Table 2)

2-amino-, 2-hydroxy-, 3-amino- and 4-amino-butyric acid were used in these studies. 2-amino-butyric acid increased γ mRNA by about threefold in the presence of 15 and 20 mM of this compound in culture and 2-hydroxy-butyric acid increased $\gamma/\gamma + \beta$ mRNA about threefold. There was essentially no increase in mRNA in the presence of 5 mM of 3-amino-butyric acid (Table 2).

4-amino-butyric acid or γ -amino-butyric acid (GABA), a compound with the amino group at a maximum distance from the carboxyl group, was tested in concentrations ranging from 0 to 30 mM and produced a small increase in $\gamma/\gamma + \beta$ mRNA ratios.

Phenyl and Methyl Derivatives of Propionic Acid (Fig. 1 and Table 3)

In these compounds, hydrophobic (phenyl and methyl) groups are present in different positions on the carbon chain of the parent compound. Three such compounds were tested: 2-phenyl-, 2-methyl-, and 3-methyl-propionic acid (Table 3).

$\gamma/\gamma + \beta$ mRNA levels increased about twofold in the presence of 2.5 mM 2-phenyl-propionic acid (Table 3). In the presence of 2 mM 2-methyl-propionic acid $\gamma/\gamma + \beta$ mRNA levels increased almost threefold, while in the presence of 2.5 mM 3-phenyl-propionic acid mRNA levels increased sevenfold. The changes in frequency of F-positive erythroblasts paralleled the changes in γ mRNA (data not shown).

Phenyl and Methyl Derivatives of Butyric Acid (Fig. 2 and Table 4)

Up to 2.5 mM of 2-phenyl-butyric acid produced only a minor increase in γ mRNA. The γ mRNA increased 2.5-fold in response to 2-methylbutyric acid (Table 4). Addition of up to 2.5 mM of 3-phenyl-butyric acid produced no significant γ mRNA induction (Table 3). In 4-phenyl-butyric acid the large phenyl group is located at the most distant position in relation to

the carboxyl group. Addition of 0.5 mM of 4-phenyl-butyric acid in primary cultures increased γ mRNA levels by more than threefold.

Esters, Amides, and Double Bonds (Figs. 1 and 2 and Table 5)

Two propionic acid esters (ethyl and methyl propionic ester) and butyramide, an amide of butyric acid, were tested in culture. These compounds share the property of the absence of the carboxyl group, which is substituted with ethyl, methyl, or amide, respectively. Our experiments investigated the role of the carboxyl group in the induction of γ -globin production *in vitro*. In addition, 2-trans-butenic acid, a compound that differs from butyric acid by a double, instead of a single, bond at position 2, was also used (Table 5).

The presence of up to 2.5 mM of propionic ethyl ester failed to induce γ -globin production at the γ mRNA level. Similarly, the addition of up to 10 mM propionic methyl ester failed to increase the $\gamma/\gamma + \beta$ mRNA ratios. Butyramide in concentrations of up to 10 mM produced a small increase in $\gamma/\gamma + \beta$ mRNA ratios, while butenoic acid (concentrations up to 2 mM) failed to increase significantly the levels of γ mRNA.

Effects on BFUe Growth

Most compounds decreased the number of BFUe colonies, as their concentrations increased. Amino derivatives appeared to have erythropoietic effects and increased colony numbers in the lower concentrations used. Thus, in 15 mM concentrations, 2-L-amino-propionic acid increased BFUe numbers to 127% of the control, while 2- and 3-amino-butyric acid produced a minor increase in the number of BFUe in 1 mM concentrations. The size of erythroid colonies was also larger compared to controls. Induction of erythropoiesis has been previously shown in BFUe cultures grown in the presence of α aminobutyric acid and *in vivo* in baboons treated with this compound (6).

Discussion

Butyrate has been shown to induce fetal hemoglobin production in primary erythroid cell cultures and *in vivo* in primates and patients (3–14). We have previously found (8) that induction of fetal hemoglobin is a property shared by all short-chain fatty acids (C₂–C₉). The most potent Hb F inducers are compounds with three, four, and five carbons. In this study, we attempted to elucidate possible chemical structure–activity relationships of these compounds. It is expected that an understanding of structure–activity relationships might help in the design of new short-chain fatty acid analogues that are Hb F inducers.

We experimented with propionic and butyric acid derivatives. Both these acids are potent fetal hemoglobin inducers (7, 8) and have small carbon chains (three and four carbons, respectively). As an assay for Hb F induction, we used BFUe cultures, as they constitute the most reliable *ex vivo* system for studying the effects of chemicals on fetal globin gene expression. Each compound was tested in BFUe cultures of cells from different donors. This approach has the disadvantage of displaying large standard deviations of the experimental results, due to the variation in Hb F induction among individuals.

A summary of the results with hydrophilic compound is provided in Figs. 3 and 4. The presence of an amino group at position 2 of propionic acid (L- and D-2-amino propionic acid) results in complete loss of the ability of propionic acid to induce fetal hemoglobin in culture. Similarly, presence of an amino group in position 2 of butyric acid results in a considerable loss of Hb F inducibility. The amino group at the end of the carbon chain (position 3 for propionic and position 4 for butyrate) results also in decreasing the Hb F-inducing potency of the original compound. The presence of a different hydrophilic group (hydroxy group) at position 2 of

butyric (2-hydroxy-butyric acid) results in a two- to threefold increase in γ -globin induction. It is likely that the presence of the hydroxy group at position 3 of propionic acid intensifies the Hb F induction potency of the hydroxy analogue, but this result should be considered very preliminary, because only one experiment was done with this compound. We also observed that in the presence of propionic and butyric acid derivatives containing amino groups, BFUe colonies grow larger in comparison to the parent compound and the hydrophobic group containing derivatives.

Results with the hydrophobic compounds are summarized in Fig. 5. Our data show that a phenyl group present close to the carboxyl group (2-phenyl-propionic, 2- and 3-phenyl-butyrate) has small effect on Hb F inducibility. In contrast, the presence of a phenyl group distal to the carboxyl group (3-phenyl-propionic and 4-phenyl-butyrate) results in potent Hb F induction, particularly by 3-phenyl-propionic acid. Similar effects are observed when a smaller hydrophobic group (methyl group) replaces the phenyl group on the carbon chain (2-methyl-propionic and butyrate). The insertion of a double bond at the molecule of butyric acid results in an almost complete loss of the Hb F-inducing properties of this compound. Propionic and butyric esters also appear to have no major effects on γ -globin induction in cultures. This may reflect the requirement of a carboxyl group for activation of γ gene expression by this family of compounds.

In summary, our results revealed the following structure–activity relationships of short-chain fatty acid derivatives: (a) In compounds bearing hydrophylic polar groups, the ability of the parent compound to induce fetal hemoglobin is reduced or completely abolished; (b) compounds bearing hydrophobic non-polar groups are in general more potent Hb F inducers; (c) the position of the substitution group seems to play a role in Hb F inducibility. The more distal to the carboxyl group, the more potent the fetal hemoglobin induction by a compound; (d) The presence of the carboxyl group seems to be a requirement for the property of fetal hemoglobin induction by short-chain fatty acids.

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Fig. 1.
Propionic acid derivatives with polar, non-polar and carboxyl substitutions used in this study.

Fig. 2.
Butyric acid derivatives with polar, non-polar and carboxyl substitutions used in this study.

Fig. 3.
The effects of hydrophilic (polar) propionic derivatives on γ -globin mRNA synthesis in human adult BFUe cultures.

Fig. 4.
The effects of hydrophilic (polar) butyric acid derivatives on γ -globin mRNA synthesis in human adult BFUe cultures.

Fig. 5.
The effects of hydrophobic (non-polar) propionic and butyric acid derivatives on γ -globin mRNA synthesis in human adult BFUe cultures.

Table 1
Effects of Amino Acid and Hydroxy Derivatives of Propionic Acid on γ -Globin mRNA Synthesis and BFUe Growth

Compound	Assays	Concentration [mM] in culture media								
		0	10	12	15	20	30	40	50	60
2-L-amino-	$\gamma\gamma + \beta$ mRNA x %	5.8 $\pm$ 2.3 (9)	5.9 $\pm$ 1.0 (3)		5.2 $\pm$ 1.0 (5)	4.3 $\pm$ 0.5 (3)	5.1 $\pm$ 1.4 (7)	4.7 $\pm$ 0.7 (5)	3.9 $\pm$ 1.3 (5)	
	Number of BFUe colonies	109 $\pm$ 70 (5)	88 $\pm$ 76 (2)		138 $\pm$ 0 (1)	93 $\pm$ 0 (1)	51 $\pm$ 7 (3)	43 $\pm$ 3 (3)	38 $\pm$ 7 (3)	
	BFUe colonies (% of control)	100	80		127	85	47	40	35	
2-D-amino-	$\gamma\gamma + \beta$ mRNA x %	5.2 $\pm$ 2.6 (8)			5.3 $\pm$ 1.0 (4)	3.5 $\pm$ 1.7 (4)	4.9 $\pm$ 2.2 (6)	3.1 $\pm$ 1.0 (4)	2.5 $\pm$ 0.5 (4)	
	Number of BFUe colonies	111 $\pm$ 57 (7)	66 $\pm$ 55 (4)		88 $\pm$ 90 (2)	77 $\pm$ 56 (3)	70 $\pm$ 31 (3)	53 $\pm$ 19 (3)	52 $\pm$ 35 (3)	
	BFUe colonies (% of control)	100	60		79	69	63	48	47	
2-hydroxy-	$\gamma\gamma + \beta$ mRNA x %	6.8 $\pm$ 4.6 (6)	6.7 $\pm$ 0.6 (3)	6.1 $\pm$ 1.9 (3)		5.5 (2)		9.5 (2)	12.3 $\pm$ 4.6 (4)	
	Number of BFUe colonies	112 $\pm$ 69 (5)	76 $\pm$ 67 (3)	100 $\pm$ 58 (2)	84 $\pm$ 60 (2)	35 $\pm$ 0 (1)		69 $\pm$ 37 (2)	53 $\pm$ 42 (2)	
	BFUe colonies (% of control)	100	68	89	75	31		62	47	
3-amino-	$\gamma\gamma + \beta$ mRNA x %	6.8 $\pm$ 3.9 (8)	7.6 (2)		6.4 $\pm$ 2.0 (5)	6.5 $\pm$ 1.8 (5)	7.4 $\pm$ 0.7 (3)	5.8 $\pm$ 1.5 (4)	7.5 $\pm$ 3.2 (4)	5.5 $\pm$ 1.0 (5)
	Number of BFUe colonies	109 $\pm$ 62 (6)	60 $\pm$ 52 (2)		112 $\pm$ 43 (2)	99 $\pm$ 56 (3)	86 $\pm$ 1 (2)	67 $\pm$ 12 (3)	50 $\pm$ 5 (3)	37 $\pm$ 6 (2)
	BFUe colonies (% of control)	100	55		102	91	78	61	46	33
3-hydroxy-	$\gamma\gamma + \beta$ mRNA x %	3.0 (1)	10.3 (1)	10.9 (1)						
	Number of BFUe colonies	NC	NC							

Note. Number of experiments in parentheses. $\pm$, standard deviations. NC, no counts.

Table 2
Effects of Amino and Hydroxy Derivatives of Butyric Acid on γ -Globin mRNA Synthesis and BFUe Growth

Compound	Assays	Concentration [mM] in culture media									
		0	1	3	5	8	10	15	20	30	40
2-amino-	$\gamma/\gamma + \beta$ mRNA x %	3.4 $\pm$ 1.5 (4)	5.9 (2)	5.1 $\pm$ 2.4 (3)		9.0 $\pm$ 3.9 (4)	8.7 $\pm$ 3.2 (4)	5.3 $\pm$ 1.4 (4)	9.3 $\pm$ 2.8 (3)	8.4 (2)	5.6 (2)
	Number of BFUe colonies	112 $\pm$ 54 (3)	120 $\pm$ 51 (2)	113 $\pm$ 54 (2)		76 $\pm$ 38 (3)	85 $\pm$ 35 (3)	82 $\pm$ 43 (3)	74 $\pm$ 36 (3)	62 $\pm$ 33 (3)	
	BFUe colonies (% of control)	100	107	101		68	76	73	66	55	
2-hydroxy-	$\gamma/\gamma + \beta$ mRNA x %	5.1 $\pm$ 2.7 (3)	10.3 $\pm$ 2.6 (3)		18.3 (2)		17.7 (2)				
	Number of BFUe colonies	90 $\pm$ 0 (2)	71 $\pm$ 8 (2)		41 $\pm$ 23 (2)						
	BFUe colonies (% of control)	100	78		45						
3-amino-	$\gamma/\gamma + \beta$ mRNA x %	3.3 $\pm$ 1.4 (4)	3.3 (2)	3.7 $\pm$ 0.9 (3)	5.1 $\pm$ 2.5 (3)	4.0 $\pm$ 0.8 (3)	4.8 $\pm$ 2.0 (3)	3.6 $\pm$ 1.8 (4)	3.9 $\pm$ 3.5 (4)		
	Number of BFUe colonies	112 $\pm$ 54 (3)	120 $\pm$ 65 (2)	89 $\pm$ 33 (2)	99 $\pm$ 41 (2)	89 $\pm$ 31 (3)	78 $\pm$ 21 (3)	80 $\pm$ 27 (3)	57 $\pm$ 29 (3)		
	BFUe colonies (% of control)	100	107	79	88	79	69	71	51		
4-amino-	$\gamma/\gamma + \beta$ mRNA x %	3.4 $\pm$ 1.5 (4)			8.1 $\pm$ 3.1 (3)	5.1 $\pm$ 2.7 (3)	4.1 $\pm$ 2.3 (4)	5.3 $\pm$ 2.7 (4)	4.7 $\pm$ 1.1 (4)	5.4 $\pm$ 1.2 (3)	
	Number of BFUe colonies	112 $\pm$ 54 (3)			100 $\pm$ 21 (2)	91 $\pm$ 15 (3)	80 $\pm$ 17 (3)	84 $\pm$ 12 (3)	80 $\pm$ 27 (3)	60 $\pm$ 21 (2)	
	BFUe colonies (% of control)	100			89	81	71	75	71	54	

Note. Number of experiments in parentheses. $\pm$, standard deviations.

Table 3
Effects of Phenyl and Methyl Derivatives of Propionic Acid on γ -Globin mRNA Synthesis and BFUe Growth

Compound (propionate)	Assays	Concentration [mM] in culture media					
		0	1.0	1.5	2.0	2.5	
2-phenyl-	$\gamma/\gamma + \beta$ mRNA x %	5.8 $\pm$ 3.5 (7)	7.6 $\pm$ 4.5 (7)	13.8 $\pm$ 7.2 (6)	11.5 $\pm$ 8.6 (7)	11.5 $\pm$ 13.6 (3)	
	Number of BFUe colonies	159 $\pm$ 97 (6)	99 $\pm$ 63 (6)	78 $\pm$ 58 (6)	61 $\pm$ 63 (5)	47 $\pm$ 47 (5)	
	BFUe colonies (% of control)	100	62	49	39	30	
2-methyl-	$\gamma/\gamma + \beta$ mRNA x %	6.0 $\pm$ 2.4	10.4 $\pm$ 2.2		16.8 $\pm$ 3.0		
	Number of BFUe colonies	112 $\pm$ 60 (5)	79 $\pm$ 51 (4)		72 $\pm$ 31 (4)		
	BFUe colonies (% of control)	100	71		65		
3-phenyl-	$\gamma/\gamma + \beta$ mRNA x %	6.6 $\pm$ 4.6 (3)	20.6 $\pm$ 9.3 (3)	30.1 $\pm$ 6.9 (3)	43.5 $\pm$ 15.7 (3)	54.6 (2)	
	Number of BFUe colonies	91 $\pm$ 50 (3)	79 $\pm$ 18 (3)	62 $\pm$ 12 (3)	54 $\pm$ 23 (3)	31 $\pm$ 12 (3)	
	BFUe colonies (% of control)	100	87	68	59	34	

Note. Number of experiments in parentheses. $\pm$, standard deviations.

Table 4
Effects of Phenyl and Methyl Derivatives of Butyric Acid on γ -Globin mRNA Synthesis and BFUe Growth

Compound	Assays	Concentration [mM] in culture media							
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
2-phenyl-	$\gamma/\gamma + \beta$ mRNA x %	7.3 $\pm$ 2.9 (3)	11.7 $\pm$ 2.4 (3)	4.9 (2)		11.0 (1)	9.8 (2)		
	Number of BFUe colonies	94 $\pm$ 13 (2)	75 $\pm$ 14 (2)	61 $\pm$ 6 (2)		50 $\pm$ 4 (2)	37 $\pm$ 1 (2)		
2-methyl-	BFUe colonies (% of control)	100	80	65		53	39		
	$\gamma/\gamma + \beta$ mRNA x %	5.1 $\pm$ 2.7 (3)		9.3 $\pm$ 5.1 (3)		9.1 (2)		13.7 (2)	12.4 (2)
	Number of BFUe colonies	90 $\pm$ 0 (2)		80 $\pm$ 12 (2)		71 $\pm$ 12 (2)		59 $\pm$ 6 (2)	63 $\pm$ 3 (2)
	BFUe colonies (% of control)	100		88		78		66	69
3-phenyl-	$\gamma/\gamma + \beta$ mRNA x %	15.2 $\pm$ 7.8 (3)		14.3 (2)	16.5 (2)		21.3 $\pm$ 11.8 (3)		
	Number of BFUe colonies	151 $\pm$ 99 (3)	64 $\pm$ 25 (2)	96 $\pm$ 82 (3)	100 $\pm$ 84 (3)	89 $\pm$ 88 (3)	68 $\pm$ 50 (2)		
4-phenyl-	BFUe colonies (% of control)	100	42	64	66	59	45		
	$\gamma/\gamma + \beta$ mRNA x %	9.1 $\pm$ 1.5 (4)	28.0 $\pm$ 8.9 (3)						
	Number of BFUe colonies	144 $\pm$ 98 (4)	82 $\pm$ 65 (4)						
	BFUe colonies (% of control)	100	57						

Note. Number of experiments in parentheses. $\pm$, standard deviations.

Table 5
Effects of Butyric or Propionic Esters and Amides on γ -Globin mRNA Synthesis and BFUe Growth

Compound	Assays	Concentration [mM] in culture media									
		0	0.1	0.2	0.5	1.0	2.0	5.0	10.0		
2- <i>trans</i> -butenoic acid	$\gamma/\gamma + \beta$ mRNA x %	5.7 $\pm$ 2.7 (3)	7.1 $\pm$ 5.0 (3)	6.7 $\pm$ 3.0 (3)							
	Number of BFUe colonies	110 $\pm$ 6 (2)	73 $\pm$ 1 (2)	58 $\pm$ 12 (2)							
	BFUe colonies (% of control)	100	66	52							
Propionic methyl ester	$\gamma/\gamma + \beta$ mRNA x %	8.1 $\pm$ 1.3				4.0 $\pm$ 1.6	5.9 $\pm$ 4.8		9.3 $\pm$ 1.6		
	F-positive erythroblasts, %	17.3					23.4				
	BFUe colonies (% of control)	100				54	49	58	26		
Propionic ethyl ester	$\gamma/\gamma + \beta$ mRNA x %	6.2 $\pm$ 3.8			5	5.6	5.1 $\pm$ 0.6				
	Number of BFUe colonies	123 $\pm$ 65 (5)			133 $\pm$ 15 (3)	87 $\pm$ 52 (5)	76 $\pm$ 0 (1)				
	BFUe colonies (% of control)	100			108	71	62				
Butyramide	$\gamma/\gamma + \beta$ mRNA x %	13.4 (2)					20.4 (2)	15.5 (2)	20.3 (2)		
	Number of BFUe colonies	91 $\pm$ 7 (3)				90 $\pm$ 43 (3)	103 $\pm$ 36 (2)	105 $\pm$ 38 (3)	71 $\pm$ 0 (3)		
	BFUe colonies (% of control)	100				99	113	115	78		

Note. Number of experiments in parentheses. $\pm$, standard deviations.