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Transcriptional regulation of *omp*F and *lam*B genetic expression by local anesthetics

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1. INTRODUCTION

In recent years local anesthetics have been used to alter the processing of precursor forms of exported proteins in *E. coli*. Whereas procaine alters the processing of few of these proteins [1,2] when it is used at high concentrations, phenethyl alcohol is much more powerful in this respect [3,4]. Both anesthetics can alter the synthesis of some of the major outer membrane proteins [2,5]. The genetic expression of the same proteins is also altered in the absence of lipid synthesis [6–8]. Since phenethyl alcohol interferes with phospholipid synthesis [9], it is interesting to extend the study of the effects of local anesthetics on genetic expression of some periplasmic and outer membrane proteins and to try to identify the targets of these drugs.

In this report, effects of procaine and phenethyl alcohol on expression of β-galactosidase in *omp*F-*lac*, *omp*C-*lac*, *lam*B-*lac* operon fusions and protein fusions, have been studied. Both compounds, in similar range of concentrations, act on this expression at the transcriptional level. Since inhibition of lipid synthesis alters the expression of β-galactosidase in the same strains at the translational level [8], the effect of both anesthetics on genetic expression of *omp*F, *omp*C, *lam*B is probably not mediated by an inhibition of lipid synthesis. Preliminary evidence suggests that one of the products of the *omp*B locus is involved in this effect.

2. MATERIAL AND METHODS

2.1. Strains and growth conditions

Bacterial strains listed in Table 1 [10–13] were generous gifts from Drs. M. Hall and M. Schwartz. Cells were grown either in a Tris-based medium of high ionic strength, supplemented with casamino acids (0.1%), thiamine and glycerol or in Luria broth of low osmotic strength as previously described [8,14]. When required, 0.2% maltose was added.

2.2. Chemicals

Phenethyl alcohol (PEA) was purchased from Fluka and procaine was obtained from Sigma Chemical Co.

2.3. Assay of β-galactosidase activity

β-Galactosidase was assayed as previously described [8].

3. RESULTS

3.1. Effects of local anesthetics on cell growth

The effects on cell growth of the various concentrations of procaine and PEA to be used in

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Table 1

List of strains

Bacterial strains *E. coli*	Genotype	Reference
MH 221-1	MH 70 Φ (*ompC-lacZ*) 10-21	[10]
MH 514	MH 20 Φ (*ompF-lacZ*) 7-14	[11]
MH 610	MH 20 Φ (*ompF-lacZ*) hyb 16-10	[11]
MH 621	MH 20 Φ (*ompF-lacZ*) hyb 16-21	[11]
pop 3098	MC 4100 Φ (*lamB-lacZ*) operon fusion	[12]
pop 3194	MC 4100 Φ (*lamB-lacZ*) hyb 52-4	[12]
pop 3105	MC 4100 Φ (*lamB-lacZ*) hyb 61-4	[13]
CW 3747	F'-lac, *met pho*R	[1]

further experiments were tested (Fig. 1). Procaine at 20 mM only increased the generation time by 30% whereas PEA at the same concentration had a dramatic effect (threefold increase). Lower concentrations for both compounds only slightly altered cell growth.

3.2. *Effects of local anesthetics on expression of β-galactosidase in* ompF- lacZ *and* lam*B*-lacZ *operon and protein fusions*

The differential rate of synthesis of β-galactosidase under the control of the *ompF* promoter was compared in cultures growing in the presence or absence of various concentrations of local anesthetics (Fig. 2). In the *ompF-lacZ* protein fusion strain (MH 610) used, as previously described [11], both transcription and translation for β-galactosidase production are under the control of *ompF* promoter. Procaine as well as PEA strongly inhibited β-galactosidase synthesis. However, as observed above for the effects on growth, the residual synthesis calculated as the ratio of slopes, to that observed with the control, was 58% and 28% for 10 mM and 20 mM procaine respectively, whereas it was 31% in the presence of only 8 mM PEA. Therefore, PEA is almost twice as efficient as procaine for inhibition of OmpF protein synthesis.

This study was extended to various operon and

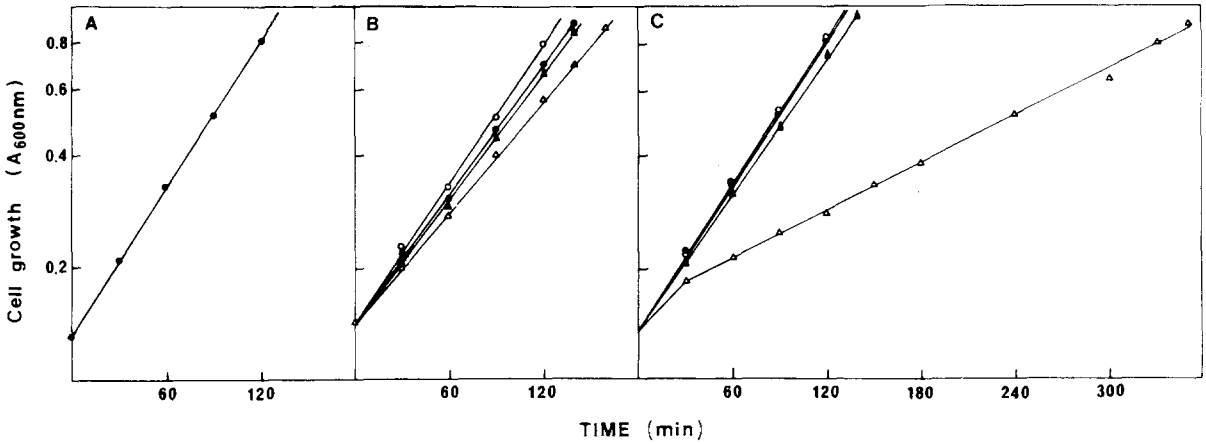

Fig. 1. Effects of local anesthetics on cell growth. Cultures of strain MH 621 were grown in minimal medium in the absence (A) or presence of procaine (B) 1 mM (○), 5 mM (●), 10 mM (▲), 20 mM (△), or of PEA (C) 0.8 mM (○), 5 mM (●), 8 mM (▲), 20 mM (△).

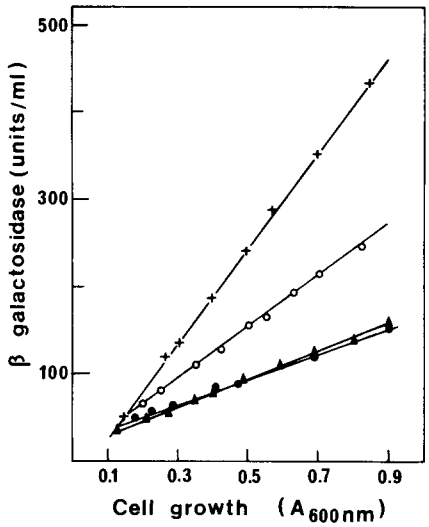

Fig. 2. Effects of local anesthetics on expression of β-galactosidase in *omp*F-*lacZ* protein fusion. A culture of strain MH 610 was grown on minimal medium to a density of $2 \cdot 10^8$ cells/ml and was then divided into 4 fractions. One fraction was kept without addition as a control (+); to the other fractions 10 mM procaine (○), 20 mM procaine (●) and 8 mM PEA (▲) respectively were added. At intervals samples were removed, the absorbance at 600 nm was measured and β-galactosidase activity was assayed.

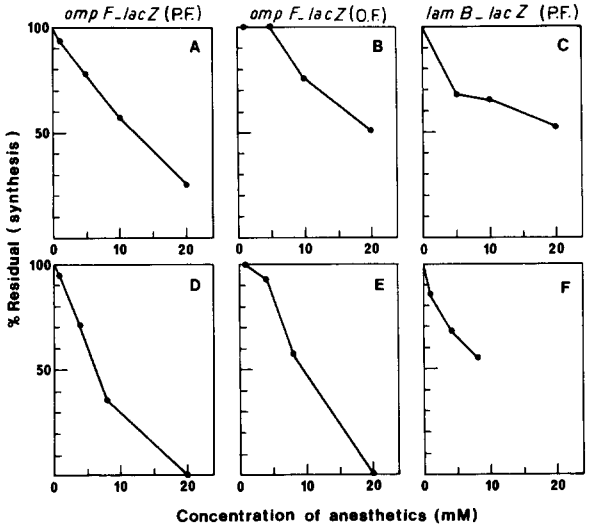

Fig. 3. Comparative effects of procaine and PEA on expression of β-galactosidase in *omp*F-*lacZ* and *lam*B-*lacZ* operon and protein fusions. β-galactosidase activity was assayed in cells grown in the presence of procaine (A, B, C) or PEA (D, E, F). The following strains were used: MH 621, (A, D); MH 514 (B, E) and pop 3194 (C, F). At the top of the figures, P.F. and O.F. stand respectively for protein fusion and operon fusion.

protein fusions (Fig. 3). By using another *omp*F-*lacZ* protein fusion (MH 621) results very similar to that described above were obtained (Fig. 3A, D). The control on β-galactosidase synthesis is probably exerted at the transcriptional level since in *omp*F-*lacZ* operon fusion a strong inhibition by local anesthetics was also observed (Fig. 3B, E), although the extent was less marked than in *omp*F-*lacZ* protein fusions.

Synthesis of LamB protein, the phage lambda receptor, was also inhibited by procaine and PEA (Table 2). This was observed both with the strain pop 3194 (Fig. 3C, F) in which the hybrid protein comprised 49 amino acid residues from pro-LamB and in strain 3105 in which the hybrid protein comprised 2 amino acids only from pro-LamB.

In contrast to the results observed after inhibition of lipid synthesis [8] that indicated no translational regulation for expression of the shorter fusion here a regulation to the same extent was exerted by both procaine and PEA at the level of transcription since the regulation was also observed in *lam*B-*lacZ* operon fusion with the strain pop 3098 (Table 2).

3.3. Effects of local anesthetics on expression of β-galactosidase in ompC-lacZ *operon fusion*

The relative amounts of OmpF and OmpC present in the outer membrane vary, depending on the growth medium. When the synthesis of OmpF is repressed, an increased production of OmpC has been evidenced [15,16]. Accordingly, we investigated the effects of local anesthetics on synthesis of β-galactosidase under the control of *omp*C pro-

Table 2

Effects of local anesthetics on expression of β-galactosidase in *lam*B-*lacZ* operon and protein fusion strains

Strains	% Residual synthesis of β-galactosidase	
	Procaine 20 mM	PEA 8 mM
pop 3098	70	80
pop 3194	52	55
pop 3105	46	48

Table 3

Effects of local anesthetics on expression of β-galactosidase in *ompC-lacZ* operon fusion (strain MH 221-1)

Medium	% Residual synthesis of β-galactosidase					
	Procaine			PEA		
	5 mM	10 mM	20 mM	0.8 mM	4 mM	8 mM
Low ionic strength	N.D.	128	133	N.D.	127	134
High ionic strength	100	100	100	105	106	110

N.D.= not determined.

moter (Table 3). An increased production of this enzyme was observed under conditions where OmpF synthesis was inhibited by local anesthetics. This was more marked when the strain was grown in low ionic strength medium, and when the inhibition of OmpF synthesis was efficient (8 mM PEA, see Fig. 3D).

3.4. Effects of ionic strength on the extent of inhibition of expression of β-galactosidase in ompF-lacZ *operon and protein fusion strains*

Since ionic strength dependent fluctuation in expression of OmpF and OmpC is mediated at the level of transcription [11], as that mediated by local anesthetics, it was of interest to determine the extent of inhibition caused by these drugs at various ionic strengths (Table 4). In both operon and protein fusions the inhibition was less marked at high ionic strength than at low ionic strength. Therefore, when the OmpF synthesis was derepressed, local anesthetics had a stronger effect.

Table 4

Effects of ionic strength on the extent of inhibition of expression of β-galactosidase in *ompF-lac* operon and protein fusion strains

Strains	% Residual synthesis of β-galactosidase			
	Procaine (10 mM)		PEA (8 mM)	
	high ionic strength	low ionic strength	high ionic strength	low ionic strength
MH 514	74	48	57	23
MH 621	54	30	32	16

3.5. Effects of local anesthetics on alkaline phosphatase synthesis

It has been previously reported that procaine alters the processing of the precursor form of alkaline phosphatase [1]; however this effect was not very marked and required high concentrations

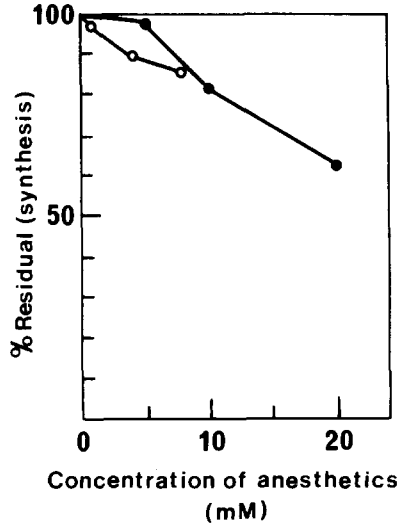

Fig. 4. Effects of local anesthetics on alkaline phosphatase synthesis. Cultures of the strain CW 3747, constitutive for alkaline phosphatase synthesis, were grown in the presence of various concentrations of procaine (●) or PEA (○). Differential rates of alkaline phosphatase synthesis were determined at each concentration of anesthetics and plotted as their ratio of the control rate observed in the absence of anesthetic.

of procaine. The main effects of local anesthetics appear to be on synthesis of this enzyme (Fig. 4) although this effect was slighter than that observed on OmpF and LamB synthesis.

4. DISCUSSION

This study was undertaken in order to compare the effects of local anesthetics on genetic expression of exported proteins with that of the inhibition of lipid synthesis previously evidenced [6–8]. The possibility of an indirect effect of anesthetics through interference with phospholipid synthesis was suggested by an earlier report showing that PEA alters this process [9].

Although it had been noticed that procaine [5] and PEA [3] can inhibit the synthesis of OmpF no quantitative evaluation of this inhibition was presented previously. A selective inhibition of synthesis of maltose binding protein by procaine was also described [17]. Since it is shown here that production of LamB is also inhibited by PEA and procaine, this suggests a coordinate inhibition of malB divergent operons products. In all cases studied, when used at the same concentration, PEA had a stronger effect than procaine. Also, the extent of inhibition was always greater in protein fusions than in operon fusions. We have no explanation for this at present. Nevertheless, since the effects were observed both in operon and protein fusions, this indicates that local anesthetics very likely act at the level of transcription. Therefore, the effects of these drugs do not appear to be mediated through an alteration of phospholipid synthesis. Since then, an inhibition at the level of translation has been evidenced [8].

The extent of inhibition of OmpF synthesis caused by procaine and PEA fluctuates depending on the ionic strength of the media. This feature suggests the involvement of one of the *ompB* products. Indeed a role for *ompB* has been demonstrated in the regulation, at the level of transcription, of the media-dependent fluctuation of OmpF. EnvZ is the product of *ompB* that is involved in this regulation [18]; this product might be the target of local anesthetics. However, this is only a possibility and more evidence is needed before it can be accepted. Regulation of alkaline phosphatase synthesis might be fitted in this hypothesis since *perA* mutant strains that are deficient in production of this protein have been shown to be equivalent to *envZ* mutants [18].

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