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Mechanism of penetration and of action of local anesthetics in *Escherichia coli* cells

Vincent Collura * and Lucienne Letellier

Laboratoire des Biomembranes, U.A. 1116 CNRS, Université Paris Sud, Orsay (France)

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***Escherichia coli* cells were used to study the mechanism of penetration of local anesthetics and the relationship between permeation and functional properties. We show that both the neutral and the protonated form of dibucaine can be accumulated in the cells. Accumulation of the protonated form occurs in response to a transmembrane electrical potential (negative inside) and results in high trapped concentrations (70 mM). Accumulation can lead to an alkalinization of the internal pH. Low concentrations of dibucaine stimulate the respiration, increase the transmembrane electrical potential and raise the accumulation of solutes. Inhibition of these functions occurs at higher concentrations of the drug. Furthermore, the drug concentration required to inhibit these functions is smaller at alkaline external pH than at acidic external pH, suggesting that the inhibition is mainly due to the neutral form of the anesthetics. Other hydrophobic amines also stimulate and inhibit different membrane functions, their efficiency being correlated to their lipophilicity.**

Introduction

Most local anesthetics are tertiary aromatic amines, the pK of which varies between 8 and 9. Therefore, they exist in both the neutral and protonated form at physiological pH. Numerous studies have shown that these drugs exert their anesthetic effect on the cytoplasmic side of excitable membranes and in the protonated form (for a review see Ref. 1). It has been assumed that the cationic form did not freely permeate into the membrane and that the drug first crossed the membrane in the neutral form, and was protonated on the inner side of the membrane [2]. Nevertheless different experiments suggest that the protonated form may also have access to the inner side of the membrane since some hydrophobic amines exert their anesthetic effect although they exist only in a protonated form at physiological pH [3]. Furthermore, Mayer et al. [4] have shown that the protonated form of dibucaine may be accumulated in large unilamellar egg phosphatidylcholine vesicles in response to a diffusion potential (negative inside). They recently confirmed this result by showing that *N*-methyldibucaine, which exists only in the cationic form, could also be accumulated in lipid vesicles in response to a diffusion potential [5].

To go further into the comprehension of the mechanism of penetration of local anesthetics into intact cells and also to relate quantitatively this permeation to the functional properties of cells, we used *Escherichia coli* cells. Respiring *Escherichia coli* cells generate an electrochemical gradient of protons:

$$\Delta\tilde{\mu}_{H^+} = F\Delta\Psi - 2.3RT\Delta pH$$

where $\Delta\Psi$ represents the transmembrane electrical potential (negative inside) and ΔpH represents the pH difference across the membrane (alkaline inside). $\Delta\Psi$ and ΔpH are easily monitored using radioactive probes [6]. Several membrane functions can be followed, allowing to correlate the changes of activity with the relative amount of protonated and neutral form of the drug. Most of the experiments described in this paper were performed with dibucaine, a commonly used local anesthetic. We show that dibucaine can be accumulated in *Escherichia coli* cells in the neutral and in the protonated form (in response to a transmembrane electrical potential) and that this permeation modifies both the

* Present address: Laboratoire d'Ingénierie des Protéines, Service des Biotechnologies, INRA, 78350 Jouy en Josas, France.

Abbreviations: α-MG, methyl α-D-glucopyranoside; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; TPP^+, tetraphenylphosphonium ion; TCS, 3,3′,4′,5-tetrachlorosalicylanilide.

Correspondence: L. Letellier, Laboratoire des Biomembranes, U.A. 1116 CNRS, Université Paris Sud, Bat 433; 91405 Orsay Cedex, France.

transmembrane electrical potential and the internal pH. Furthermore, depending on the relative concentrations of the charged and neutral form, dibucaine either enhances or inhibits several cellular functions. Some of these effects are compared to those induced by other related drugs.

Materials and Methods

Materials

[^{3}H]TPP$^+$, [^{14}C] sucrose, [^{14}C] benzoate and tritiated water were purchased from CEA, France. [^{14}C]Lactose, [^{14}C]α-MG, ^{86}Rb and [^{14}C]proline were from Amersham, U.K. All materials were of reagent grade and were obtained from commercial sources.

Growth conditions and cell treatment

Escherichia coli cells from the strain ML 308–225 (i^-, z^-, y^+, a^+) were grown aerobically at 37°C in minimal synthetic medium, supplemented with 0.4% glycerol (v/v). Bacteria were harvested by centrifugation at an absorbance of 0.5 at 650 nm.

Cells were permeabilized to the membrane potential probe TPP$^+$, by a treatment with Tris-EDTA [7]. The cells, treated or not with EDTA were centrifuged and washed once with sodium phosphate buffer 100 mM at the desired pH, and containing 1 mM KCl. They were then resuspended in the same buffer at an $A_{650} = 50$ (25 mg cell dry weight/ml), kept on ice and used within 3 h. All assays were performed at 20°C in this buffer.

Uptake of dibucaine in Escherichia coli cells

Dibucaine was assayed by fluorescence ($\lambda_{ext} = 327$ nm, $\lambda_{em} = 410$ nm) in a quartz cuvet of 1 cm pathlength. The cell suspension (5 ml, 2.5 mg dry weight/ml) energized by the addition of glycerol (0.4%) and oxygenated by addition of catalase (5000 U/ml) and H_2O_2 (0.53 mM) was incubated at 20°C for 15 min, in the presence of dibucaine at the desired concentration. This time was sufficient to obtain a steady state level of accumulation of dibucaine. (not shown). The suspension was then centrifuged for 3 min on a microfuge TH12 and the supernatant was carefully collected. After appropriate dilution, the fluorescence of the supernatant was measured and the concentration was calculated by reference to a standard sample of anesthetic containing glycerol, catalase and H_2O_2. The entrapped dye concentration was obtained from the difference between these two fluorescences. The uptake of dibucaine in deenergized cells was measured under similar conditions except that the cells and the control were treated with the protonophore TCS (10 μM, final concentration) 5 min prior to the addition of dibucaine. TCS was prefered to CCCP since it does not interfere with the fluorescence of dibucaine. The values given in the text are the result of at least nine independent experiments.

Determination of the cytoplasmic volume

The cytoplasmic volume of the dibucaine-treated cells and of the control cells was determined with ^3H_2O (0.12 MBq/ml) as described in Ref. 6. [^{14}C]Sucrose (0.6 μM, 20 GBq/mmol), which passes through the outer membrane but is excluded by the inner membrane, was used as an external marker [6]. Catalase (5000 U/ml) and H_2O_2 (0.53 mM) were added to prevent anaerobiosis. [^{14}C]-Sucrose was purified before use, to eliminate glucose contamination. For this purpose glucose grown ML 308 225 cells (5 mg dry weight/ml) were incubated during 10 min in the presence of [^{14}C]sucrose (0,04 mM, 20 GBq/mmol). The cells were centrifuged and the supernatant containing the purified sucrose was used immediately after. The volume was determined using 1.5 ml of the cell suspension (5 mg cell dry weight/ml) and using the centrifugation technique.

Determination of ΔpH

ΔpH was estimated from the accumulation of [^{14}C]-benzoate (1.5 μM, 19.7 GBq/mmol) in the presence of tritiated water (0.12 MBq/ml) as a marker of the total water content [6]. The cells (1 mg dry weight/ml) were incubated for 15 min with dibucaine in the assay medium. Then, the radioactive probes, catalase (5000 U/ml) and H_2O_2 (0.53 mM) were added. The method of separation was centrifugation.

Measurement of ΔΨ and of the initial rate of lactose influx

ΔΨ was determined from the accumulation of [^{3}H] TPP$^+$. Energized EDTA-treated cells (1 mg dry weight/ml) were suspended in the assay medium and incubated with agitation for 15 min in the presence of dibucaine. The cells were further incubated during 10 min with 10 μM [^{3}H] TPP$^+$ (3.7 GBq/mmol). [^{14}C] lactose (0.1 mM, 36 MBq/mmol) was then added and aliquots (100 μl) were filtered 10 s later on Whatman glass microfiber filters (GF/F), and washed twice with 4 ml of the assay medium. The filters were counted for radioactivity on the preset ^{3}H/^{14}C program. The filtrations were made in triplicate and TPP$^+$ uptake was corrected for nonspecific binding by subtracting a blank obtained under the same conditions except that the cells, incubated in the presence or in the absence of dibucaine, were pretreated with 10 μM TCS.

Measurement of ΔΨ with $^{86}Rb^+$ (0.5 μM, 37 MBq/mg) was performed in a similar way except that the cells were treated with 10 μM valinomycin and that no KCl was added to the buffer.

Initial rate of α-MG and proline influx

[^{14}C] α-MG (0.1 mM, 5.55 GBq/mmol) or [^{14}C]-proline (0.1 mM, 9.25 GBq/mmol) were added to 100 μl of the cell suspension containing dibucaine and the sample was filtered 10 seconds later as described above.

Rate of respiration

Oxygen consumption was determined polarographically with a Gilson oxygraph using a Clark oxygen electrode. The cell concentration was 1 mg dry weight/ml.

ATP measurement

The energized cells (1 mg dry weight/ml), were incubated in the presence or in the absence of dibucaine in the assay medium for 15 min. 10 µl of cell suspension were diluted with 90 µl dimethylsulfoxide; 4.9 ml of sterile H_2O was then added to prevent any interference of dimethylsulfoxide in the luminescence assay. 100 µl of this suspension were withdrawn for ATP measurement with a luminometer (Lumac) using the luciferin/luciferase assay system.

Results

Effect of dibucaine and other related drugs on the transmembrane electrical potential and internal pH of Escherichia coli cells

Fig. 1 compares the effects of increasing concentrations of dibucaine on the transmembrane electrical potential for two values of the external pH : 6.6 and 8. Under these conditions dibucaine which has a pK of 8.8 [8] is essentially protonated at pH 6.6 and 84% protonated at pH 8. ΔpH values were measured in a parallel experiment (Fig. 2). In the absence of drug, the values of $\Delta\Psi$ and ΔpH were, respectively, 160 mV and 0.75 pH unit at $pH_{ext} = 6.6$, and 200 mV and 0 pH unit at $pH_{ext} = 8$. Addition of low concentrations of dibucaine significantly enhanced $\Delta\Psi$ (30 to 40 mV). The range of concentrations for which $\Delta\Psi$ was enhanced decreased with increasing external pH, and thus with the increasing amount of neutral form of the anesthetics. The further increase of drug concentration resulted in a progressive decrease of $\Delta\Psi$, which was even collapsed when the drug concentration exceeded 2 mM at $pH_{ext} = 8$. Fig. 2 shows that there was a more pronounced

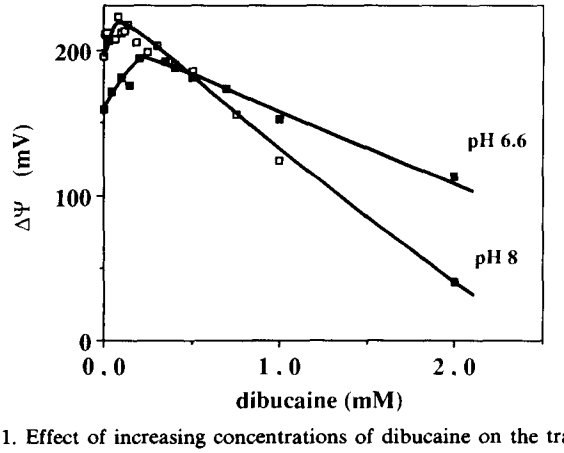


Fig. 1. Effect of increasing concentrations of dibucaine on the transmembrane electrical potential of EDTA-treated *Escherichia coli* cells. $\Delta\Psi$ was measured with [^{3}H]TPP$^+$ at pH 6.6 (■) and 8 (□). Dibucaine was added 15 min before TPP$^+$. A mean value of 1.2 µl/mg cell dry weight was taken for the cytoplasmic volume.

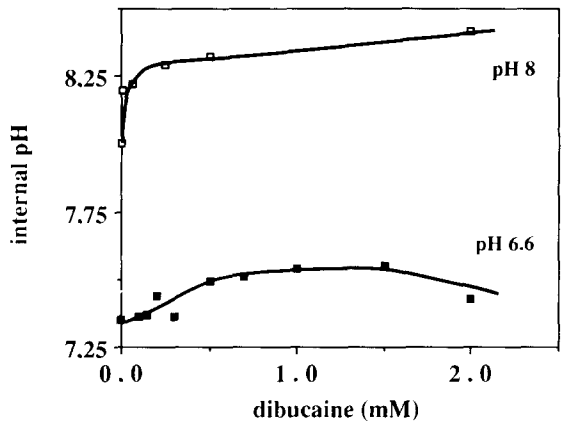
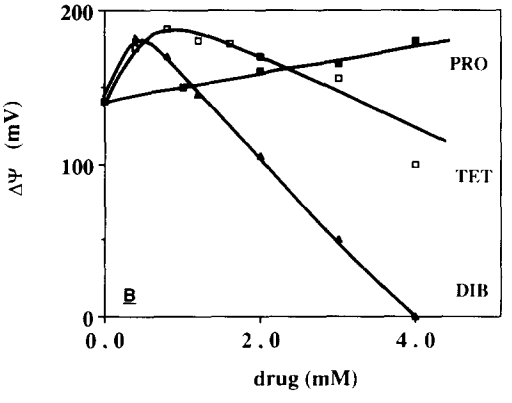


Fig. 2. Internal pH of control and dibucaine-treated cells. EDTA-treated cells were incubated with dibucaine during 15 min prior to the addition of [^{14}C]benzoate. The external pH was 6.6 (■) or 8 (□). Values are given with an error bar of 0.05 pH unit.



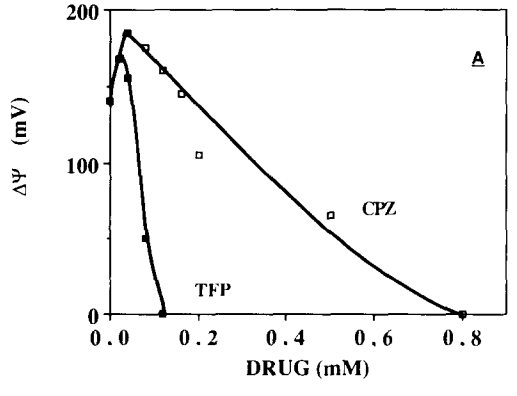


Fig. 3. Effect of various drugs on the transmembrane electrical potential of *Escherichia coli* cells. Experimental conditions were identical to those described in legend to Fig. 1 except for the pH of the buffer which was 7.2. (A) Chlorpromazine (CPZ, □) trifluoroperazine (TFP, ■). (B) Procaine (PRO, ■) tetracaine (TET, □) dibucaine (DIB, ▲)

TABLE I

Comparison of the effects of different hydrophobic amine drugs in bacteria and mitochondria

References are given in square brackets. n.d., not determined.

Drug	pK	Concn. (mM) [a]	Uncoupling efficiency [b]	log P [c]
Trifluoroperazine	n.d.	0.08	n.d.	n.d.
Chlorpromazine	9.3 [10]	0.5	1000	5.28
Dibucaine	8.8 [8]	2.8	99.5	4.29
Tetracaine	8.5 [9]	6	11.9	3.73
Procaine	9 [10]	> 40	40	1.90

[a] Concentration of drug required to decrease $\Delta\Psi$ to 60 mV in *Escherichia coli* cells incubated at pH 7.2 (see Fig. 3).
[b] As determined in mitochondria (from Ref. 12).
[c] P is the octanol/water partition coefficient (taken from Ref. 12).

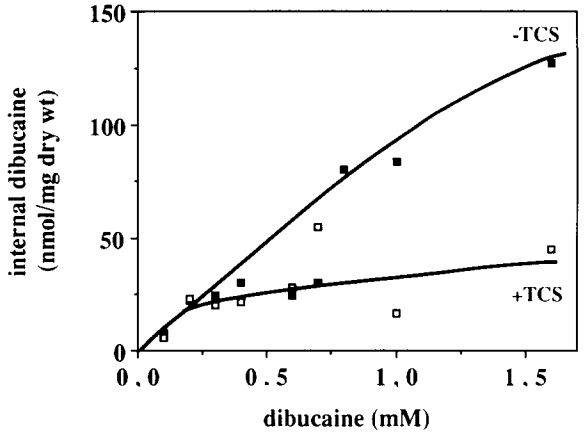


Fig. 4. Accumulation of dibucaine in energized and deenergized *Escherichia coli* cells. The amount of trapped dibucaine was determined by fluorescence as described in Materials and Methods. The cells incubated at pH 6.6 were treated (□) or not (■) with 10 μM TCS prior to the addition of dibucaine.

alkalinisation of pH_{in} (0.4 unit) when the cells were incubated at $pH_{ext} = 8$ (i.e., when the drug was partially in the neutral form) than at $pH_{ext} = 6.6$ (i.e., when the drug was almost fully protonated).

The same $\Delta\Psi$ changes were obtained when $^{86}Rb^+$ in the presence of valinomycin was used instead of $[^{14}C]TPP^+$, thus excluding a possible dibucaine-induced artefact in the $\Delta\Psi$ measurement (data not shown). The variations of $\Delta\psi$ and ΔpH could not be attributed to a change of internal volume which was not significantly different in the presence of dibucaine (1.36 and 1.29 ± 0.05 μl/mg dry weight at pH 6.6 and 8, respectively) and in the absence of drug (1.13 ± 0.05 μl/mg). A mean value of 1.2 μl/mg was used in all calculations.

Fig. 3 represents the $\Delta\Psi$ changes induced by various drugs carrying an aromatic backbone and an amine function for which the pK varies between 8.5 (tetracaine) and 9.3 (chlorpromazine) (Table I and Refs. 9, 10). All the drugs induced a large increase of $\Delta\Psi$ (from 145 to 190 mV) followed by a decrease. Nevertheless the concentration required to abolish $\Delta\Psi$ differed among the drugs, and concentrations of procaine up to 20 mM were not sufficient to decrease $\Delta\Psi$ (Table 1).

Uptake of dibucaine in energized and deenergized cells

The uptake of dibucaine in *Escherichia coli* cells was measured for two different values of pH_{ext} using the fluorescence technique. At $pH_{ext} = 6.6$ and in the range of concentrations tested, the $\Delta\Psi$ of energized cells varied between 130 and 190 mV (Fig. 1) and ΔpH did not change significantly (Fig. 2). Fig. 4 shows that the amount of drug trapped was higher in energized cells (125 ± 15 nmol dibucaine/mg dry weight) than in cells treated with the protonophore TCS ($\Delta\Psi$ and $\Delta pH = 0$) (40 ± 15 nmol/mg dry weight). At pH 8, only a small range of dibucaine concentrations was tested since the drug induced an important decrease of $\Delta\Psi$ for concentrations larger than 1 mM (see Fig. 1). In the range of dibucaine concentrations tested (from 0 to 0.5 mM)

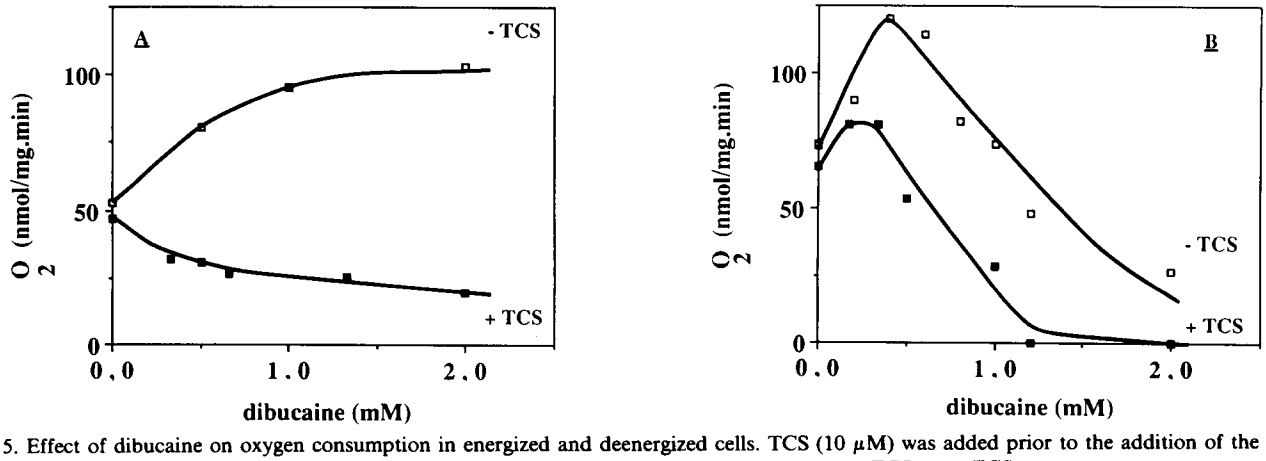


Fig. 5. Effect of dibucaine on oxygen consumption in energized and deenergized cells. TCS (10 μM) was added prior to the addition of the drug. The experiments were performed at pH 6.6 (A) and pH 8 (B). ■, +TCS; □, −TCS.

there was no appreciable difference in the amount of trapped drug in energized and deenergized cells (30 ± 15 nmol/mg dry weight) (data not shown).

The respiratory activity of dibucaine-treated cells

The respiratory activity was measured at pH 6.6 and pH 8 in energized and deenergized (TCS-treated) cells. In the absence of dibucaine, the respiratory activity was the same whatever the state of energization (figs. 5A and B). These results are in good agreement with those reported previously [11] which showed that *Escherichia coli* lacks respiratory control, i.e., the respiratory activity is not increased in the presence of protonophore. Increasing the external pH from 6.6 to 8 in these control cells resulted only to a 10 to 20% increase of the respiratory activity. Addition of dibucaine strongly affected the respiratory activity. In energized cells, low concentrations of dibucaine stimulated the respiration and high concentrations inhibited it partially. A similar behaviour has also been observed with several local anesthetics in mitochondria [12,13]. The concentration required to inhibit the respiration was smaller at alkaline pH (> 0.5 mM) than at acidic pH (> 2 mM). Furthermore, the concentration at which the respiratory activity started to decrease was higher than that required to decrease $\Delta\Psi$ (see Fig. 1). The effect of dibucaine on TCS-treated cells was more complex and strongly dependent on pH_{ext}: at pH 8, the respiratory activity first increased at low dibucaine concentration and then decreased. At pH 6.6, the respiratory activity was inhibited over the whole range of dibucaine concentrations. An intermediate situation was observed at pH 7.2: the respiratory activity remained constant on a wide range of dibucaine concentrations (0 to 2.5 mM) and fell to zero beyond 2.5 mM (data not shown).

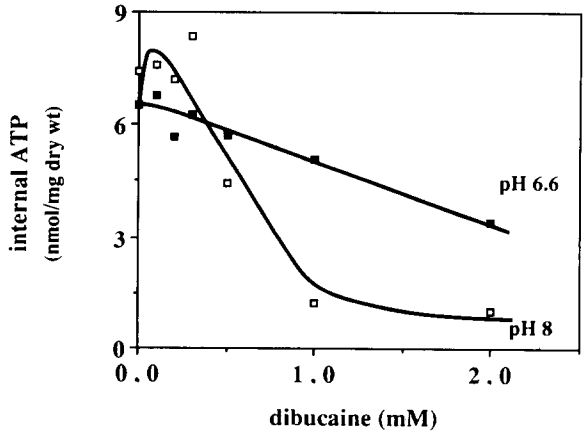


Fig. 6. Effect of dibucaine on the internal ATP concentration of energized *Escherichia coli* cells. ATP was measured 15 min after the addition of dibucaine as described in Materials and Methods. The pH of the buffer was either 6.6 (■), or 8 (□).

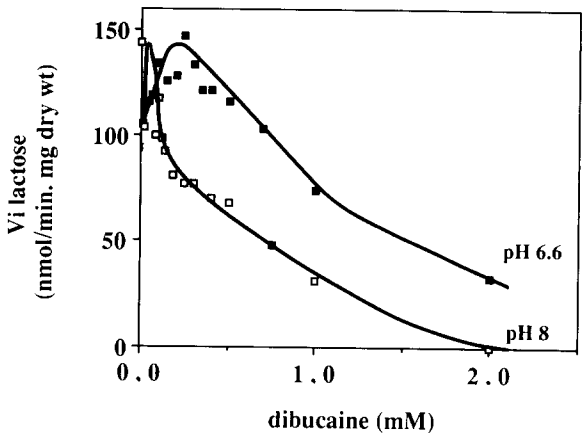


Fig. 7. Effect of dibucaine on the initial rate of lactose uptake in *Escherichia coli* cells. Energized cells were incubated either at pH 6.6 (■) or pH 8 (□) in the presence of dibucaine and for 15 min. Then lactose (0.1 mM, final concentration) was added. Initial rates were measured in triplicate 10 s after lactose addition.

ATP content of the dibucaine treated-cells

Intracellular ATP slightly increased in the presence of small dibucaine concentrations and decreased at higher concentrations (Fig. 6). This decrease occurred at lower concentrations and was more pronounced at pH 8 than at pH 6.6.

Solute uptake in the presence of dibucaine

Fig. 7 shows the effect of dibucaine on the active transport of lactose which is cotransported with protons [14]. The changes of the initial rate (V_i) of lactose transport evolved in parallel with those of $\Delta\Psi$ (see Fig. 1): it increased at low dibucaine concentration and decreased at higher concentrations.

Table II shows that other lipophilic amine drugs were also able to stimulate the rate of lactose influx. Furthermore these drugs also stimulate the transport of other solutes: proline (a sodium and H^+ coupled active transport) [15] and methyl α-D-glucopyranoside, a non

TABLE II

Relative rates of solute accumulation in Escherichia coli cells treated with local anesthetics

The initial rates (V_i) of lactose, proline and α-MG transport were determined as described in Material and Methods. The external solute concentration was 0.1 mM. The local anesthetics were added 15 min prior to the solutes.

Drug	V_i lactose	V_i proline	V_i α-MG
None	1	1	1
Dibucaine 0.2 mM	1.3 ± 0.1	1.9 ± 0.2	n.d
Tetracaine 0.25 mM	1.3 ± 0.1	2.7 ± 0.2	2 ± 0.1
Procaine 5 mM	1.5 ± 0.1	n.d.	n.d.

metabolizable analog of glucose which is transported via the PTS system [16].

Discussion

We have first provided evidence that the electrochemical gradient of protons can drive the accumulation of the protonated form of local anesthetics in bacteria. Indeed, at pH 6.6, the amount of accumulated drug (which is essentially in the protonated form) was higher in energized cells (125 nmol dibucaine/mg dry weight) than in cells treated with the protonophore TCS ($\Delta\Psi$ and $\Delta pH = 0$) (40 nmol/mg dry weight). It is unlikely that this accumulation may be driven by the pH gradient (which is about 1 unit) since its direction (more alkaline inside) is unfavorable to an accumulation (one expects that at equilibrium $[DibH^+]_{in}/[DibH^+]_{out} = [H^+]_{in}/[H^+]_{out}$). We thus favour the hypothesis that the protonated drug is accumulated in response to the transmembrane electrical potential (negative inside). Under conditions where $\Delta\Psi$ and $\Delta pH = 0$, one can assume that the concentration of free dibucaine in the cytoplasm is equal to the external one. Thus, an external concentration of 1 mM would correspond to a free internal concentration of 1.2 nmol/mg dry weight (a value calculated using a cytoplasmic volume of 1.2 μl/mg dry weight). Since about 40 nmol/mg were trapped in deenergized cells, this suggests that most of the drug is associated with the membranes. Fig. 4 showed that the amount of drug trapped in deenergized cells did not increase when the concentration of dibucaine was increased above 0.25 mM; this suggests that the number of dibucaine binding sites is saturable. If one assumes that the number of binding sites is identical in energized and deenergized cells, then the concentration of dibucaine accumulated in response to $\Delta\Psi$ can be calculated from the difference between the concentration of drug trapped in energized and deenergized cells. A concentration of 70 mM is found. It should be stressed that such high concentrations of dibucaine are also accumulated in liposomes in response to a diffusion potential [5]. Fig. 2 showed that a large alkalinization of pH_{in} occurred when the drug was partially deprotonated ($pH_{ext} = 8$) whereas pH_{in} remained practically constant when the drug was almost fully protonated ($pH_{ext} = 6.6$). This suggests that the deprotonated form of the drug, which is freely permeable across membranes [17], is mainly responsible for the alkalinization.

Interestingly, small concentrations of dibucaine and of other related drugs enhance significantly $\Delta\Psi$. This increase is not the consequence of the interconversion of ΔpH into $\Delta\Psi$ [18] since the ΔpH was not significantly modified at $pH_{out} = 6.6$. Although the hypothesis of a decrease of passive leak cannot be ruled out since it is known that the interaction of local anesthetics with lipid head groups modifies the permeability of membranes [19–21] it is more lileky that the increase of $\Delta\Psi$ may be due to the large stimulation of the respiratory activity.

In energized cells the stimulation of the respiration occurred over a broader range of dibucaine concentration when the drug was fully protonated (pH 6.6) than when both forms (protonated and neutral) were present (pH 8), suggesting that the inhibition is related to the increase of the neutral form of the anesthetic. The effect of dibucaine on deenergized cells (TCS-treated) was also strongly pH dependent. At pH 8 the stimulation and the inhibition of the respiration occurred in the same range of concentrations as for energized cells. Surprisingly, at pH 6.6, we observed that the respiration was inhibited over the whole range of concentrations. Clearly, the acidification of the external medium is not responsible for the inhibition of respiration, since this inhibition was not observed in energized cells. On the other hand, in the presence of the protonophore, the internal pH of the cells has decreased from 7.25 to 6.6. We thus favour the hypothesis that, in the presence of dibucaine, this acidification of pH_{in} affects one or more components of the respiratory chain on the cytoplasmic side of the membrane.

Small concentrations of dibucaine also had a strong stimulating effect on several functions:. The higher ATP content of dibucaine-treated cells is probably related to the increase of $\Delta\Psi$ but one cannot exclude a direct interaction of the drug with the ATPsynthase as suggested by the work of Vanderkooi et al. on the isolated F_1-ATPase of mitochondria [22]. The enhancement of lactose transport may be attributed to the increase of $\Delta\Psi$ since the V_{max} of lactose transport is known to be a function of $\Delta\Psi$ [23,24]. The same argument may prevail in the case of proline

The results described above suggest that the toxicity of local anesthetics in *Escherichia coli* increases with increasing amount of the neutral form of the drug. Indeed, smaller concentrations of dibucaine were required to abolish $\Delta\Psi$ and to impair the different functions at alkaline pH than at acidic pH. The effect, on $\Delta\Psi$, of various drugs having similar pK values but different lipophilicities (see Table I) suggests that their toxicity increases with increasing lipophilicity. Interestingly a good correlation exists between the effect of these drugs in *Escherichia coli* and in mitochondria (see Table I and Ref. 12). Thus, the general statement that the toxicity of local anesthetics is related to their lipophilicity and to the amount of neutral form also prevails in bacteria. The results presented here also show that the protonated form of local anesthetics can be accumulated in whole cells in response to a membrane potential. Although very high internal concentrations of the drug can be attained, the cells retain, at least partially, their functionality. This may be of relevance for

the mechanism of action of these drugs in excitable membranes.

Local anesthetics are known to prevent the export of *Escherichia coli* periplasmic and outer membrane proteins. As a result, the precursors are generally accumulated in the cells [25–28]. It was generally admitted that the local anesthetic would induce structural alterations of the membranes (changes in fluidity) which would in turn be responsible for the accumulation of precursors. Most of these experiments were performed with concentrations of drugs which we show here to be sufficient to depolarize the cells and to decrease the ATP content. Since the export of bacterial proteins requires energy in the form of ATP and of electrochemical gradient of protons [29] this suggests that the accumulation of precursors induced by local anesthetics is probably the result of the perturbation of the energetic state of the bacteria.

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References

1 Hille, B. (1980) in Molecular Mechanisms of Anesthesia, (Fink, B., ed.), Vol 2, Raven Press, New York.
2 Courtney, K.R. and Strichartz, G.R. (1987) in Local Anesthetics, Handb. Exp. Pharm., Vol. 81 (Strichartz, G.R., ed.), pp. 54–94, Springer Verlag, Heidelberg.
3 Lee, A.G. (1978) Biochim. Biophys. Acta, 514, 95–104.
4 Mayer, L.D., Bally, M.B., Hope, M.J. and Cullis, P.R., (1985) J. Biol. Chem. 260, 802–808.
5 Mayer, L.D., Wong, K.F., Menon, K., Chong, C., Harrigan, P.R. and Cullis, P.R. (1988) Biochemistry 27, 2053–2060.
6 Rottenberg, A. (1979) Methods Enzymol. 55, 547–569.
7 Boulanger, P. and Letellier, L. (1988) J. Biol. Chem. 263, 9767–9775.
8 De Jong, R.H. (1977) in Local Anesthetics, III, p. 281, Charles C. Thomas, Springfield, IL.
9 Ritchie, J.M. and Greengard, P. (1961) J. Pharm. Exp. Ther., 133, 241–245.
10 Green, A.L. (1967) J. Pharmac. Pharmacol. 19, 10–16.
11 Burstein, C., Tiankova, L. and Kepes, A. (1979) Eur. J. Biochem., 94, 387–392.
12 Garlid, K. and Nakashima, R., (1983) J. Biol. Chem. 258, 7974–7980.
13 Dabadie, P., Bendriss, P., Erny, P. and Mazat, J.P. (1987) FEBS Lett. 226, 77–82.
14 West, I.C. and Mitchell, P. (1973) Biochem. J. 312, 587–592.
15 Chen, C., Tsuchiya, T., Yamane, Y., Wood, J.M. and Wilson, T.H. (1985) J. Membr. Biol. 84, 157–164.
16 Postma, P.W. and Lengeler, J.W. (1985) Microbiol. Rev. 49, 232–269.
17 Schreier, S., Fregatti, W.A., Aranjo, P.S., Chaimovich, H. and Cuccovia, I.M. (1984) Biochim. Biophys. Acta, 769, 231–237.
18 Booth, I.R., Mitchell, W.J. and Hamilton, W.A. (1979) Biochem. J. 182, 687–696.
19 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504–519.
20 Seelig, A., Allegrini, P.R. and Seelig, J. (1988) Biochim. Biophys. Acta 939, 267–276.
21 Ohki, S., (1984) Biochim. Biophys. Acta 777, 56–99.
22 Vanderkooi, G., Shaw, J., Storms, C., Vennerstrom, R. and Chignell, D. (1981) Biochim. Biophys. Acta 635, 200–203.
23 Geck, P. and Heinz, E. (1976) Biochim, Biophys. Acta 443, 49–63.
24 Ghazi, A. and Shechter, E. (1981) Biochim. Biophys. Acta 644, 305–315.
25 Tribhuwan, R.C. and Pradhan, D.S. (1977) J. Bacteriol. 131, 431–437.
26 Halegoua, S. and Inouye, M. (1979) J. Mol. Biol. 130, 39–61.
27 Pages, J.M. and Lazdunski, C. (1981) C.R. Acad. Sci. 293, 2730.
28 Pugsley, A.P., Conrad, D.J., Schnaitman, C.A. and Greeg, T.I. (1980) Biochim. Biophys. Acta 599, 1–12.
29 Eilers, M. and Schatz, G. (1988) Cell 52, 481–483.