JOURNAL OF BACTERIOLOGY, Aug. 1984, p. 605–610
0021-9193/84/080605-06$02.00/0
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Vol. 159, No. 2

Voltage Clamp Effects on Bacterial Chemotaxis

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Received 23 February 1984/Accepted 8 May 1984

To examine whether or not sensory signaling in bacteria is by way of fluctuations in membrane potential, we studied the effect of clamping the potential on bacterial chemotaxis. The potential was clamped by valinomycin, a K^+-specific ionophore, in the presence of K^+. Despite the clamped potential, sensory signaling did occur: both *Escherichia coli* and *Bacillus subtilis* cells were still excitable and adaptable under these conditions. It is concluded that signaling in the excitation and adaptation steps of chemotaxis is not by way of fluctuations in the membrane potential.

Whether or not membrane potential ($\Delta\psi$) is involved in chemotaxis of peritrichous bacteria is an unresolved question. On the one hand, changes in the level of $\Delta\psi$ affect the swimming pattern of these bacteria (10, 11, 27, 41, 44, 45); consequently, a distinct $\Delta\psi$ signaling protein was postulated (33), lated called a protometer, a putative proton motive force (PMF) sensor with a role in aerotaxis and phototaxis (see, e.g., reference 62 for review and references cited therein). On the other hand, electrical signaling seems unlikely in view of the recent observations of Ishihara et al. (26) that sensory response in filamentous cells of *Escherichia coli* has a finite range. One of the ways to distinguish between response to a potential change and signaling by way of fluctuations in $\Delta\psi$ is to clamp the potential at a constant value and study the effect of this clamping on the response of the bacteria to a chemical stimulant. A clamped potential can be achieved with valinomycin, a K^+-specific ionophore (5, 53), in the presence of external K^+. Such an approach has been applied successfully to nonperitrichous bacteria, in which chemotaxis of *Spirochaeta aurantia* (20, 21) and phototaxis of *Rhodospirillum rubrum* (24) were inhibited by this treatment. Measurements of the chemotactic response of peritrichous bacteria have been published for a *Streptococcus* sp., in which a clamped potential did not prevent the response to the attractant leucine (41). In this paper, we show for the peritrichous bacteria *E. coli* and *Bacillus subtilis* that both the excitation and adaptation processes of chemotaxis still occur under conditions where $\Delta\psi$ is clamped.

MATERIALS AND METHODS

Chemicals. [^{14}C]tetraphenylphosphonium bromide was obtained from Amersham Corp., and ^3H_2O, [^{14}C]benzoate, and [^{14}C]inulin were from New England Nuclear Corp. L-Serine was from ICN Pharmaceuticals. Indole, α-*methyl*-DL-aspartate, and valinomycin were from Sigma Chemical Co. Nigericin was from Calbiochem. All other chemicals were of the highest purity commercially available.

Bacteria. The *E. coli* strain used in this study, RP487 (23), is a K-12 derivative and was obtained from J. Adler (University of Wisconsin—Madison). *B. subtilis* OI1085 (66) was received from G. W. Ordal (University of Illinois, Urbana). Both strains are wild type for chemotaxis. The *E. coli* strain was grown in tryptone broth as previously described (50). The cells were permeabilized by the Tris-EDTA technique of Leive (36) as modified by Szmelcman and Adler (61). The efficiency of the permeabilization was determined as described previously (12). Only batches in which at least 99.9% of the population was permeabilized were used. The permeabilized cells were finally suspended in a chemotaxis medium (pH 6.0) consisting of phosphate buffer (50 mM), glycerol (5 mM), EDTA (0.1 mM), and L-methionine (0.1 mM for temporal assays and 1 μM for capillary assays). *B. subtilis* was grown in nutrient broth (Difco Laboratories) as described previously (27), washed three times, and resuspended in a chemotaxis medium consisting of KP_i (50 mM), $(NH_4)_2SO_4$ (0.3 mM), $CaCl_2$ (0.14 mM), glycerol (0.1 M), DL-lactate (10 mM), and EDTA (0.1 mM) (final pH, 6.0). This chemotaxis is a slight modification of the one used in previous studies (27, 46).

Chemotaxis assays. Capillary assays were performed as described by Adler (1). Permeabilized cells of *E. coli* were suspended in the chemotaxis medium described above to a concentration of 12.5 μg of protein per ml (optical density at 590 nm, 0.05). Each assay was incubated for 45 min at 30°C. Temporal assays (39), based on the procedure described by Goy et al. (23), were carried out at room temperature (26°C) as follows. At time zero, the attractant was added to a 50-μl suspension of bacteria in chemotaxis medium in a tube. At various intervals thereafter, a 5-μl sample was removed, placed on a microscope slide, and mixed with 5 μl of 1 mM indole, a potent repellent of *E. coli* (65). The tumbling frequency of the cells was monitored and recorded. The adaptation time was defined as the first time, after the addition of the attractant, at which indole-stimulated tumbling occurred. In control experiments, 5 μl of chemotaxis medium was added instead of indole. In these temporal assays we had to employ indole rather than repellents such as acetate or benzoate, which cause much longer duration of tumbling (e.g., see Table 5 in reference 51 for the response times observed with these repellents), because the latter two, or any other weak organic acid, cannot be used in combination with valinomycin. Such a combination totally dissipates the PMF, with concomitant paralysis of the cells (see below).

Measurement of PMF. Measurement of PMF was carried out at room temperature (26°C) by the centrifugation technique, using [^{14}C]tetraphenylphosphonium (final concentration, 1 μM; 8.3 nCi/ml) as a $\Delta\psi$ probe, [^{14}C]benzoate (15 μM; 0.4 μCi/ml) as a $pH_{in} - pH_{out}$ (ΔpH) probe, and [^{14}C]inulin (0.5 mg/ml; 2.5 μCi/ml) and ^3H_2O (7 μCi/ml) as markers for the external and total water space, respectively (55). The

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bacteria were suspended in chemotaxis medium to a concentration of 0.6 mg of protein per ml (final pH, 6.0). The values of $\Delta\psi$ and PMF given were corrected for nonspecific binding of tetraphenylphosphonium to the membrane, using cells in the presence of the uncoupler carbonyl cyanide-*p*-(trifluoromethoxy)-phenylhydrazone (50 μM). These values are imprecise due to the uncertainty involved in quantitative corrections for binding to energized cells (2, 5, 38, 63, 67).

Ionophore-treated cells. Valinomycin and nigericin were dissolved in ethanol and added in a volume not exceeding 0.05%. The final concentration of valinomycin was 20 nmol/mg of protein and that of nigericin was 13 nmol/mg of protein (in capillary assays) or 1 μM (in temporal assays and in PMF measurements).

Since the outer membrane must be permeable for these ionophores to be effective, and since the outer membrane may be resealed in the presence of glycerol or other energy sources (37), the bacteria were periodically checked for maintenance of their permeability. In addition, each batch of bacteria in 50 mM KP_i buffer was periodically checked to affirm loss of motility in the presence of both valinomycin (dissipates $\Delta\psi$) and 40 mM benzoate (dissipates ΔpH). In rare cases, we had batches of permeabilized bacteria with ΔpH values close to zero. In these bacteria, the addition of valinomycin dissipated the PMF almost completely, and consequently, their swimming stopped. These batches, therefore, were not used, as well as other strains that we have examined (e.g., AW405 [4] or AW620 [32]), in which valinomycin caused either biased smooth swimming (see reference 27) or paralysis. (We have indeed found that $\Delta\psi$ in strain AW405 [-131 ± 4 mV] was larger than in RP487 [-112 ± 9 mV], and this may explain the more drastic effect of valinomycin on AW405 than on RP487. It is also possible that in RP487 the reduction of motility with concomitant suppression of tumbling occurs only at a relatively low value of PMF. It should be emphasized that inhibition of motility could cause artifacts in capillary assays. Thus, when the motility was much reduced by valinomycin, e.g., in strain AW405, the inhibitory effect of valinomycin was larger in attractant-filled capillaries than in attractant-free capillaries, probably because of the biased smooth swimming under conditions of reduced PMF.)

Addition of benzoate alone (in 50 mM KP_i suspensions) or nigericin alone (in 2 mM KP_i plus 48 mM Na_2P_i suspensions) caused a repellent response due to the drop in internal pH (29, 54); the cells tumbled for several minutes and then the original tumbling frequency was restored (see references 27, 29, 45, 54).

RESULTS

Valinomycin and nigericin effects on PMF and swimming behavior. Studying the effect of valinomycin, at high external K^+ concentrations, on the behavior of *E. coli* has several difficulties. (i) The outer membrane of *E. coli*, a gram-negative organism, is impermeable to valinomycin (12, 47). (ii) At neutral pH, the membrane potential is the major component of the PMF (47, 58), the driving force for flagellar rotation (8, 14, 19, 28, 31, 40, 41, 42, 52, 64). Dissipation of $\Delta\psi$ by valinomycin may, therefore, abolish motility. (iii) Lowering the PMF even to a value above the threshold for motility may perturb the observation by suppression of tumbling (27). These difficulties were overcome or circumvented by (i) permeabilizing the outer membrane of the bacteria by Tris-EDTA treatment, (ii) carrying out the experiments at pH 6.0, at which the PMF is considered to be above the threshold for motility even when $\Delta\psi$ is dissipated (the threshold value is estimated to be about 0.2 to 0.6 pH units [8, 28, 52, 57]), and (iii) choosing a bacterial strain (RP487) in which reduction of motility with concomitant suppression of tumbling does not occur even when $\Delta\psi$ is dissipated. The effects of valinomycin were compared with those of nigericin, an ionophore which at low concentrations transports K^+ for H^+ electroneutrally and thus predominantly affects ΔpH (5, 53). Valinomycin totally dissipated $\Delta\psi$ in a chemotaxis medium containing 50 mM KP_i, and nigericin only slightly reduced the ΔpH (Table 1). The small effect of nigericin under the present conditions is probably because the chemical gradients of both ions, K^+ and H^+, are inwardly directed. (The internal K^+ concentration in permeabilized *E. coli* is ca. 20 mM [6]). The measured values of ΔpH in RP487 were relatively low because of the preceding treatment to permeabilize the cells (cf. reference 54 for ΔpH values in permeable cells of *E. coli*). The value of ΔpH obtained with nonpermeable cells, in the same experimental solution as in Table 1, was 1.6 ± 0.1 pH units, in accordance with previously published values (47, 58).

To examine if indeed the potential was clamped by valinomycin, we compared the effect of the attractant L-serine on $\Delta\psi$ in the presence and the absence of the ionophore. The membrane potential was parallelly monitored by two fluorescent dyes with different electrical charges and different mechanisms of response to $\Delta\psi$ (see reference 17). L-Serine caused a hyperpolarization of 5 mV (see reference 12) only when valinomycin was absent (Fig. 1A and C). In the presence of valinomycin, no change in $\Delta\psi$ was observed (Fig. 1B and D).

The permeabilization itself caused some decrease in the speed of swimming of the bacteria, but did not affect their swimming mode (Table 2). Valinomycin under these conditions did not further affect either the speed or mode of swimming of RP487 cells having a sufficiently large ΔpH, nor did nigericin. This is in agreement with previous studies of *B. subtilis* (28, 45) and *Streptococcus* sp. (41).

Valinomycin effects on chemotaxis. The effect of valinomycin on the chemotactic response of the bacteria in a medium containing 50 mM KP_i is shown in Table 3. Since it was difficult to accurately distinguish the transition between wild-type-like swimming and smooth swimming, and vice versa, we determined the response to the attractant and the duration of the response (= adaptation time in the table) by periodic additions of the repellent indole subsequent to the addition of the attractant (see above). Even under conditions of clamped and dissipated $\Delta\psi$, the cells were stimulated by the attractant L-serine and then adapted. The threshold concentration was not affected by valinomycin, but the adaptation time in some experiments seemed to be shorter in the presence of valinomycin. We had similar observations with α-*methyl*-DL-aspartate, a non-metabolizable attractant

TABLE 1. Valinomycin and nigericin effects on $\Delta\psi$ and ΔpH of permeabilized cells of *E. coli* in KP_i buffer[a]

Ionophore added	$\Delta\psi$ (mV)	ΔpH	PMF (mV)
None	-112 ± 9	0.6 ± 0.3	-151 ± 28
Valinomycin	-5 ± 9	0.5 ± 0.2	-38 ± 23
Nigericin	-104 ± 18	0.4 ± 0.1	-126 ± 23
Valinomycin + nigericin	0	0.3 ± 0.2	-20 ± 11

[a] The chemotaxis medium in which the bacteria were suspended contained 50 mM KP_i buffer. The values given are the averages of 9 determination $\pm$ the standard deviation. Temperature = 26°C.

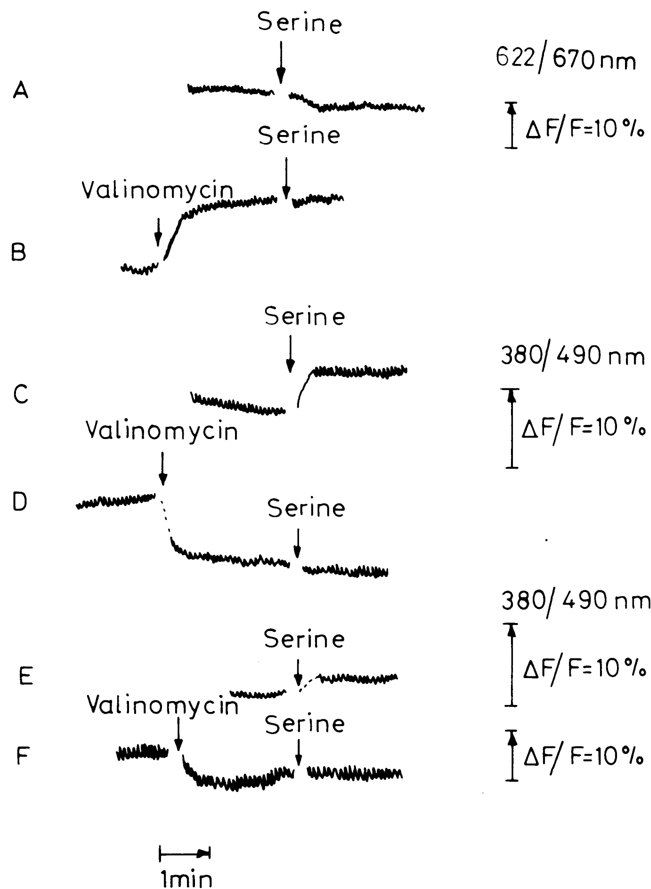

FIG. 1. Valinomycin effect on serine-stimulated changes in $\Delta\psi$ of *E. coli*. The fluorescence measurements were carried out in thermostated cuvettes (30°C) with a Perkin-Elmer MPF-44A fluorometer as previously described (17). The excitation/emission wavelengths were 622/670 nm and 380/490 nm (slit width, 10 nm) for 3,3'-dipropylthiodicarbocyanine iodide and 1-anilinonaphthalene-8-sulfonate, respectively. The dye concentrations were 0.1 μM (3,3'-dipropylthiodicarbocyanine iodide) and 40 μM (1-anilinonaphthalene-8-sulf0nate). The experiments were carried out with a bacterial concentration of 75 μg of protein per ml in chemotaxis medium that also contained 2 mM amytal and 20 μM *N,N'*-dicyclohexylcarbodiimide (see reference 12). The dye-containing suspensions were allowed to equilibrate with the inhibitors for 10 min before L-serine (10 μM) or valinomycin was added. (A and B) Fluorescence changes of 3,3'-dipropylthiodicarbocyanine iodide in high-potassium buffer (50 mM KP_i). (C and D), Fluorescence changes of 1-anilinonaphthalene-8-sulfonate in high-potassium buffer. (E and F), Fluorescence changes of 1-anilinonaphthalene-8-sulfonate in low-potassium buffer (2 mM KP_i plus 48 mM NaP_i). Note that with 3,3-dipropylthiodicarbocyanine iodide a decrease in fluorescence means hyperpolarization, whereas with 1-anilinonaphthalene-8-sulfonate an increase means hyperpolarization.

of *E. coli* (43; data not shown in the table). We also had similar observations with *B. subtilis*, a gram-positive bacterium whose cytoplasmic membrane is accessible to valinomycin without any pretreatment (11, 44); addition of L-serine (1 mM) to strain OI1085 caused smooth swimming for 1.1 ± 0.2 min and 0.7 ± 0.1 min in the absence and presence of valinomycin, respectively.

TABLE 2. Valinomycin and nigericin effects on the swimming behavior of *E. coli* in KP_i buffer[a]

Cells and treatment	Speed of swimming (μm/s)[b]	Cell proportion (%)[c]		
		S	w.t.	T
Nonpermeable cells	30 ± 9	28 ± 9	58 ± 6	14 ± 3
Permeable cells	20 ± 7	20 ± 5	61 ± 1	19 ± 6
Permeable cells + valinomycin	22 ± 5	24 ± 10	57 ± 12	19 ± 3
Permeable cells + nigericin	19 ± 5	31 ± 4	51 ± 3	18 ± 1

[a] The experimental conditions were as described in Table 1.
[b] The values given are average values ± standard deviation (the average sample size was ca. 30 bacteria).
[c] The values given are averages of one to three fields ± the standard deviation. Sample size in each field was ca. 40 bacteria. S, w.t., and T stand for smooth, wild-type-like, and tumbling modes of swimming, respectively.

Valinomycin and nigericin effects in low-K^+ buffer. Valinomycin in the presence of 50 mM KP_i (equivalent to ca. 55 mM K^+ at pH 6.0) not only clamps, but also dissipates, the potential. To examine the effect of a clamped but not dissipated potential on the swimming behavior of the bacteria, we repeated the experiments of Tables 1 through 3 but in chemotaxis medium containing 2 mM KP_i plus 48 mM NaP_i. Since in the absence of K^+ valinomycin does not affect the PMF (3, 17, 47) or the swimming behavior of the bacteria (Table 4), it was hoped that in the presence of only 2 mM K^+ (lower than the presumed internal concentration [6]) valinomycin would clamp $\Delta\psi$ without dissipating it. This was the observed result (Table 4). Nigericin, on the other hand, reduced ΔpH more effectively under these conditions than in the presence of 50 mM KP_i (Table 1), probably due to the outwardly directed chemical gradient of K^+. As a result of this reduction, $\Delta\psi$ was increased, and consequently, the total PMF was practically unaffected by nigericin (see, e.g., references 41, 49, and 54 for similar observations and reference 3 for a different observation). Valinomycin clamped the potential at the value shown in the table; nigericin did not increase the potential in the presence of valinomycin. Furthermore, L-serine-stimulated hyperpolarization could not be detected in the presence of valinomycin (Fig. 1E and F).

The effects of valinomycin on the swimming behavior of the bacteria were similar in either 2 or 50 mM KP_i; the threshold concentration of L-serine was the same under conditions of clamped potential as of nonclamped potential, and the adaptation time was reduced on the average by 24%. Nigericin did not have or had very small effect on the

TABLE 3. Valinomycin effects on temporal assays to L-serine in KP_i buffer[a]

Ionophore added	Threshold concn (μM)[b]	Adaptation time (min)[c]
None	2–4	1.9 ± 0.4
Valinomycin	2–4	1.5 ± 0.3

[a] The experimental conditions were as described in Table 1, and the experimental procedure is described in the Materials and Methods section.
[b] The threshold concentration is defined here as the lowest attractant concentration needed to prevent indole-stimulated tumbling. The values given are from four different experiments. The attractant concentration was examined in threefold increments.
[c] L-Serine concentration was 10 μM. The values given are the averages of five determinations ± the standard deviation.

TABLE 4. Valinomycin and nigericin effects on the PMF, swimming behavior, and chemotactic response in low-K^+ buffer[a]

Ionophore added	$\Delta\psi$ (mV)[b]	ΔpH[b]	PMF (mV)[b]	Speed of swimming (μm/s)[c]	Cell proportion (%)[d]			Threshold concn (μM)[e]	Adaptation time (min)[f]
					S	w.t.	T		
None	−115 ± 12	0.9 ± 0.2	−171 ± 22	23 ± 4	24 ± 5	61 ± 5	15 ± 6	2–4	1.7 ± 0.3
Valinomycin	−107 ± 18	0.7 ± 0.1	−152 ± 25	24 ± 4	19 ± 12	62 ± 10	19 ± 8	2–4	1.3 ± 0.2
Nigericin	−141 ± 2	0.4 ± 0.1	−166 ± 10	26 ± 5	25 ± 0	57 ± 6	18 ± 6	0.8–4	2.1 ± 0.2
Valinomycin + nigericin	−101 ± 8	0.4 ± 0.1	−125 ± 11	26 ± 6	17 ± 1	59 ± 1	24 ± 2	ND[g]	ND
None[h] (50 mM NaP_i)	ND	ND	ND	25 ± 5	20 ± 2	71 ± 6	9 ± 4	ND	1.2 ± 0.2
Valinomycin[h] (50 mM NaP_i)	ND[i]	ND	ND	26 ± 3	20	68	12	ND	1.1 ± 0.2

[a] The experimental conditions were as described in Table 1, except that the medium contained 48 mM NaP_i plus 2 mM KP_i buffer, instead of 50 mM KP_i.
[b] The values given are the averages of 6 to 12 determinations ± the standard deviation.
[c] The values given are the average values ± standard deviation.
[d] The values given are the averages of 10 fields ± the standard deviation, excluding lines 3 and 6 in which the values given are for 2 fields and 1 field, respectively. Sample size, ca. 30 bacteria in each field. S, w.t., and T stand for smooth, wild-type-like, and tumbling modes of swimming, respectively.
[e] The values given are from two different experiments. The attractant concentration was examined in two- to threefold increments. In the given concentration range, the response to indole was partial. Below and above this range there was full or no response to indole, respectively.
[f] The values given are the averages of 12 determinations ± the standard deviation. L-Serine concentration was 10 μM.
[g] ND, Not determined.
[h] These permeable cells were resuspended in 50 mM NaP_i buffer instead of 48 mM NaP_i + 2 mM KP_i.
[i] Using a tetraphenylphosphonium-selective electrode or a K^+-selective electrode (see reference 17), we have determined that valinomycin does not affect $\Delta\psi$ in permeabilized *E. coli* cells suspended in K^+-free NaP_i buffer. The apparent values of $\Delta\psi$ were not, however, determined in those experiments.

threshold concentration of L-serine. However, it seemed to increase the average adaptation time.

We also examined the effects of the ionophores on chemotaxis judged by capillary assays. Neither valinomycin nor nigericin had a significant effect on the number of bacteria accumulated in L-serine-filled capillaries in a 2 mM KP_i plus 48 mM NaP_i medium (Table 5).

DISCUSSION

The mechanism of chemotaxis in peritrichous bacteria involves two major steps, excitation and adaptation (25, 48, 60). The excitation process transduces the initial signal from the receptor to the flagella and affects the direction of their rotation. The adaptation process restores the unstimulated behavior of the bacteria. In view of (i) the relatively fast excitatory signaling in *E. coli* (≦200 ms [56]), (ii) the analogy to much larger bacterial species, for which evidence in favor of electrical signaling has been shown (7, 9, 18, 20–22, 30, 35), and (iii) the observation that an external electric field affects chemotaxis of *E. coli* (16), the notion of electrical signaling in peritrichous bacteria seemed feasible. This notion was, however, questioned by Synder et al. (59), who calculated that a period of 200 ms is sufficient for signaling even by way of molecular diffusion, and by Ishihara et al. (26), who found that the internal signal for changing the direction of flagellar rotation has a shorter range than would be expected for electrical signaling.

This study shows that transduction of sensory signals in *E. coli* and *B. subtilis* is not by way of fluctuations in $\Delta\psi$. Both the excitation and adaptation processes did not occur under conditions of a clamped potential (Tables 3 and 4). These observations thus endorse the finding of Manson et al. (41) that *Streptococcus* sp., when powered by a diffusion potential in the presence of valinomycin, still shows a chemotactic response to leucine, and extend it to *E. coli* and *B. subtilis*, powered by respiration.

Our observation that clamping the potential did not prevent excitation seems to be in line with the suggestion of Snyder et al. (59) and Ishihara et al. (26) that sensory signaling is not electrical in nature. Based on our study, this conclusion is, however, restricted only to fluctuations in $\Delta\psi$, and only if the diffusion of the valinomycin-K^+ complex across the membrane is faster than the hypothesized fluctuations in $\Delta\psi$. This seems reasonable. Assuming 4 nm as the width of the cytoplasmic membrane and the diffusion constant of the valinomycin-K^+ complex to be of the order of 3 $\times 10^{-8}$ cm^2/s, one may calculate that it would take ca. 3 μs for the complex to diffuse from one side of the membrane to the other and thus to clamp the potential (see reference 34). Changes in $\Delta\psi$ that occur in a shorter time range seem very unlikely.

Our experimental conditions did not allow the determination of the delay response time of the bacteria to the stimulants, so we could not deduce whether the duration of the excitatory sensory transduction was affected by the clamped potential. In spite of this drawback, the conclusion reached above holds.

The adaptation time was decreased by clamping the potential and increased by the increasing $\Delta\psi$ (or reducing ΔpH) in the presence of nigericin (Tables 3 and 4). Due to the

TABLE 5. Valinomycin and nigericin effects in capillary assays[a]

Ionophore added	Threshold concn (M)[b]	Peak concn (M)[c]	Avg no. of accumulated bacteria in capillaries containing:	
			No attractant	Serine (10 mM)
None	10^{-6}	10^{-2}	1,400 ± 200	60,000 ± 7,000
Valinomycin	10^{-5}	10^{-2}	3,600 ± 1,100	110,000 ± 30,000
Nigericin	10^{-6}	10^{-2}	2,500 ± 1,200	52,000 ± 1,000

[a] The experimental conditions were as described in the Materials and Methods section. The phosphate buffer was 48 mM NaP_i plus 2 mM KP_i.
[b] The threshold concentration is defined here as the lowest concentration of L-serine that gives an accumulation in the capillary greater than that obtained in the absence of attractant (1). The serine concentration was examined in 10-fold increments.
[c] The peak concentration is defined here as the concentration of L-serine in the capillary in which the maximal number of bacteria were accumulated (1).

relatively small changes in adaptation time, we analyzed the statistical significance of these changes by Student's *t* test. Only the changes in low-K^+ buffer (Table 4) were statistically significant. The probability that a real change in adaptation time occurred in the presence of the ionophore was 99.9 and 97% for valinomycin and nigericin, respectively. The statistical significance of the change in adaptation time in high-K^+ buffer (Table 3) or with *B. subtilis* was, however, doubtful ($P \leq 90\%$). Since the average change in adaptation time was only 24%, it seems to us that this change may be the consequence of a secondary or an indirect effect of $\Delta\psi$ on one of the processes involved in adaptation, e.g., the chemotaxis-related hyperpolarization (12, 13, 15, 17) (cf. Fig. 1). Thus, a clamped potential may facilitate the cation efflux that is possibly correlated with the adaptation time (M. Eisenbach, T. Raz, and A. Ciobotariu, unpublished data) and in this way accelerate the adaptation process. An increased $\Delta\psi$ may slow down this efflux and thus increase the adaptation time. Other possible explanations cannot, however, be ignored. The clamped potential may reduce the magnitude of the excitation signal or the dynamic range for excitation, or it may shift the definition of what is an adapted cell with a resultant shorter "distance" between the adapted and nonadapted states. Our observation that macroscopic chemotaxis assays showed no significant inhibition in spite of the change in the adaptation time (provided that motility was not impaired) seems to support the notion that these changes in adaptation time are secondary or indirect effects.

Our conclusion, with *E. coli* and *B. subtilis*, against sensory transduction by way of fluctuations in $\Delta\psi$ may not hold in nonperitrichous bacteria. Recently Goulbourne and Greenberg (20, 21) reported that a voltage clamp inhibited the accumulation of *S. aurantia* in attractant-filled capillaries. On the basis of this finding and their observation that neurotoxins (that affect the action potential in eucaryotic cells) also inhibited accumulation in the capillaries (22), they concluded that $\Delta\psi$ fluctuations or ion fluxes are involved in the transduction of sensory signals. It appears, therefore, that in *S. aurantia*, unlike *E. coli* or *B. subtilis*, sensory transduction may be related to fluctuations in $\Delta\psi$.

ACKNOWLEDGMENTS

We thank R. Chen and A. Ciobotariu for carrying out capillary assays, and J. Adler, H. C. Berg, S. R. Caplan, E. P. Greenberg, and R. M. Macnab for their constructive comments on this manuscript.

This study was supported by research grants from the U.S. National Institute of Allergy and Infectious Diseases, from the U.S.-Israel Binational Science Foundation, Jerusalen, Israel, and from the Fund for Basic Research administered by the Israel Academy of Sciences and Humanities. M.E. holds the Barecha Foundation Career Development Chair.

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