A Process Related to Membrane Potential Involved in Bacterial Chemotaxis to Galactose†

Michael Eisenbach,* Tirza Raz, and Adina Ciobotariu

ABSTRACT: Attractants, in the presence of respiration and ATPase inhibitors, stimulate a hyperpolarization in *Escherichia coli* [Eisenbach, M. (1982) *Biochemistry 21*, 6818–6825]. In order to examine whether this hyperpolarization is correlated with chemotaxis, the effect of the attractant D-galactose and its analogues on the membrane potential of wild-type *E. coli* strains and some of their mutants was studied. The main observations were the following: (i) Wild-type cells became hyperpolarized by either galactose or its nonmetabolizable analogues, D-fucose and L-sorbose. (ii) A mutant defective in galactose metabolism became hyperpolarized by galactose. (iii) Inhibiting the galactose permease system did not prevent the hyperpolarization, rather it facilitated the observation of the hyperpolarization. (iv) Mutants unable to transport galactose via the methyl β-galactoside (Mgl) transport system but having normal chemotaxis to galactose became normally hyperpolarized by D-fucose. (v) Mutants which cannot bind galactose were not hyperpolarized by galactose. (vi) The hyperpolarization in *flaI* mutants, in which the whole chemotaxis machinery is repressed, was reduced to 12–15% of the hyperpolarization in the parent strains. (vii) Nonattractant sugars did not stimulate hyperpolarization. It is concluded that the hyperpolarization is the consequence of neither galactose metabolism nor transport but rather is correlated with galactose taxis.

Changes in the membrane potential of *Escherichia coli* stimulated by attractants have been followed by several research groups, the goal being to determine if the membrane potential is involved in chemotaxis (e.g., Szmelcman & Adler, 1976; Armitage & Evans, 1981; Snyder et al., 1981; Goulbourne & Greenberg, 1981). Previous results and conclusions had been in apparent disagreement until it was recently shown that the discrepancies were the consequence of differing experimental conditions (Eisenbach, 1982). Processes that are involved in membrane potential production may mask those changes in the membrane potential that are possibly correlated with chemotaxis. Inhibiting these processes (e.g., respiration by amytal or KCN) allowed the detection of an apparent hyperpolarization stimulated by attractants (Eisenbach, 1982). An unsolved question in our previous study was the nature and significance of this hyperpolarization. Choosing D-galactose as a representative attractant (Adler, 1969), we recently showed that the apparent hyperpolarization is a true change in the membrane potential rather than a response of the measuring probe (TPP^+)[1] to surface charge changes (M. Eisenbach, Y. Margolin, A. Ciobotariu, and H. Rottenberg, unpublished results). In the present study we used the same attractant, D-galactose, to study the functions involved with this hyperpolarization.

The galactose-binding protein of *E. coli*, a protein that has been isolated and well-characterized (Anraku, 1968a,b; Boos, 1972, 1974; Boos & Gordon, 1971; Zukin et al., 1977; Miller et al., 1980; Quiocho & Pflugrath, 1980; Mahoney et al., 1981), serves two independent functions. The binding of D-galactose to this protein triggers chemotaxis toward galactose (galactose taxis) and the transport of galactose across the bacterial cytoplasmic membrane via the methyl β-galactoside transport system (Hazelbauer & Adler, 1971). In principle, any process correlated with these two functions or with the metabolism of galactose may be involved in the observed changes in the membrane potential. It is the goal of the present work to identify this process.

Experimental Procedures

Chemicals. D-[U-^{14}C]Galactose (318 Ci/mol) was obtained from Amersham and [^{3}H]TPP^+Br^- (2.5 Ci/nmol) from Nuclear Research Center, Negev (Israel). Nonradioactive TPP^+Br^- was synthesized as previously described (Eisenbach, 1982). Glucose-free D-galactose and D-fucose were obtained from Sigma. D-Fucose and L-sorbose were purified by Dr. Arye Tishbee (The Weizmann Institute of Science) by using high-performance liquid chromatography with a Lichrosorb-NH_2 (10 μm) 4.6 × 250 mm column, refraction index detector, and mobile phase of 25% (v/v) acetonitryl in water. The sugars were examined for purity by paper chromatography (Putman, 1957; Adler, 1969). All other chemicals were of the highest purity commercially available.

Bacteria. The strains used in this study are listed with their relevant genotypes in Table I. Before use, they were characterized on tryptone swarm plates and on galactose minimal swarm plates (Adler, 1966a). The bacteria were grown and prepared for the experiments as previously described (Eisenbach, 1982).

Measurement of Membrane Potential. Measurements of membrane potential were carried out by monitoring the external TPP^+ concentration in a suspension of permeabilized bacteria with a TPP^+-specific electrode, as previously described in detail (Eisenbach, 1982). All measurements were carried out at 30 °C. Amytal (2 mM) and DCCD (20 μM) were added at least 10 min before the addition of the attractant. This combination of inhibitors was chosen to allow correction for the nonspecific TPP^+ binding, based on the anoxia at the

† From the Department of Membrane Research, The Weizmann Institute of Science, 76100 Rehovot, Israel. *Received February 11, 1983.* This work was supported by research grants from the U.S. National Institute of Allergy and Infectious Diseases and from the U.S.–Israel Binational Science Foundation (BSF), Jerusalem, Israel. M.E. holds the Barecha Foundation Career Development Chair.

[1] Abbreviations: ATPase, adenosinetriphosphatase; DCCD, *N,N'*-dicyclohexylcarbodiimide; FruDP, D-fructose 1,6-bisphosphate; MCP, methyl-accepting chemotaxis protein; Mgl, methyl β-galactoside; TPP^+, tetraphenylphosphonium; wt, wild-type; ΔE, change in electrode potential; $\Delta\psi$, membrane potential; $\Delta\tilde{\mu}_{H^+}$, proton electrochemical potential (in mV). Both $\Delta\psi$ and $\Delta\tilde{\mu}_{H^+}$ are defined to be positive for an outwardly directed potential gradient (inside positive). Hyperpolarization means, in this study, an increase in the absolute value of $\Delta\psi$ or that $\Delta\psi$ becomes more negative.

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Table I: *E. coli* Strains Used in This Study

strain[a]	relevant genotype or phenotype	parent strain	reference
AW546	wt	B275	Hazelbauer & Adler (1971)[b]
AW550	*mglB* class I[c]	AW546	Hazelbauer & Adler (1971), Boos et al. (1981)
B275	wt	F3-W1-6[d]	Adler (1966b)
AW541	*mglB* class II[c]	B275	Hazelbauer & Adler (1971)
AW543	*mglB* class II[c]	B275	Hazelbauer & Adler (1971)
OW1[e]	wt	AW574[f]	Ordal & Adler (1974a)
OW11	*mglB* class III[c]	OW1	Ordal & Adler (1974a,b)
OW15	*mglA*	OW1	Ordal & Adler (1974a,b)
OW24	*mglC*	OW1	Ordal & Adler (1974a,b)
AW493[g]	wt		Fraenkel (1968), Adler (1969)
DF2000	Gal$^-$Glc$^-$	AW493	Fraenkel (1968), Adler (1969)
HB233[h]	wt	RP437	Hazelbauer & Engström (1981)
HB261	*flaI*	HB233*eda*$^+$	Hazelbauer & Engström (1981)
AS-1	wt	W3110	Hirota et al. (1981)
As-1*flaI*[i]	*flaI*	As-1*eda*$^+$	
RP437	wt	RP461	Parkinson (1978)
RP3098	Δ(*flaI-flaH*)	RP437	Parkinson & Houts (1982)

[a] All strains, but AW493 and DF2000, are *E. coli* K12 derivatives. Excluding HB233 and HB261 which were obtained from Dr. G. L. Hazelbauer, all other strains were received from Dr. J. Adler. [b] AW546 appears in this reference as B275*his*$^-$. [c] The classes of *mglB* mutants are those defined by Ordal & Adler (1974b), according to their galactose taxis and galactose transport properties. Some of these properties are described in Table IV. [d] F3-W1-6 is a derivative of the Lederberg strain W1 (Adler, 1966b). [e] OW1 was obtained by Ordal & Adler (1974a) by crossing AW574 with AB1927. [f] AW574 (Tso & Adler, 1974) is a *gal*$^+$*su*$^-$ isolate of AW405 (Armstrong et al., 1967). [g] AW493 is strain K10, a prototrophic Hfr, a wild-type parental strain for DF2000. [h] HB233 is RP437*met*$^+$. [i] AS-1*flaI* was constructed by J. Pierce in J. Adler's laboratory, by P1 transduction of the *flaI* mutation from *flaI*am88 (Kondoh et al., 1980) into AS-1*eda*$^+$, using *eda* as a selective marker.

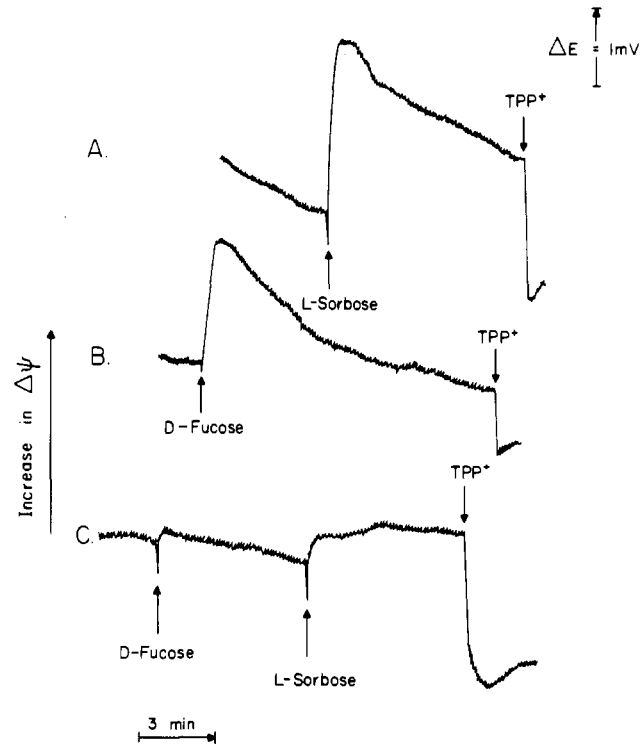

FIGURE 1: Changes in membrane potential stimulated by nonmetabolizable analogues of D-galactose. The membrane potential was monitored by the TPP^+ electrode as described under Experimental Procedures. The strain used was AW546. Prior to the addition of D-fucose or L-sorbose, the reaction mixture contained 1.5 mL of suspension of strain AW546 (0.35 mg of protein/mL) in 10 mM KP_i, 5 mM $MgSO_4$, 2 mM amytal, 0.1 mM EDTA, 0.1 mM L-methionine, 20 μM DCCD, and 5 μM TPP^+ (final pH 7.0; temperature = 30 °C). Purified L-sorbose (62 μL to form a final concentration of 8.3 mM) (A) or purified D-fucose (30 μL to form 5.2 mM) (B) was added where indicated. As a control, 30 μL of D-fucose and 65 μL of L-sorbose (final concentrations 5.2 and 8.3 mM, respectively) were added to heat-treated bacteria (C). At the end of each experiment 1 μL of 1 mM TPP^+ was added for calibration. (Note the differences in the calibration response between the traces.)

end of the experiment (Eisenbach, 1982). The calculated values of the membrane potential should be regarded with caution due to the uncertainty in the correction for the adsorption of TPP^+ to the bacterial membrane (Ahmed & Booth, 1981; Ten Brink et al., 1981; Zaritsky et al., 1981; Bakker, 1982; Lolkema et al., 1982). In order to measure the membrane potential simultaneously with galactose uptake we used the filtration technique (Schuldiner & Kaback, 1975) with [^{3}H]TPP^+Br^- and D-[^{14}C]galactose.

Results

A hyperpolarization, stimulated by galactose, could, in principle, be the consequence of any of the following processes: aerobic or anaerobic metabolism of galactose, ATP hydrolysis via the ATPase, transport of galactose, or chemotaxis toward galactose. Under our experimental conditions [i.e., in the presence of respiratory and ATPase inhibitors (Eisenbach, 1982)], we have already shown that respiration and ATP hydrolysis via the ATPase are not involved in the observed hyperpolarization. The other parameters will be individually examined.

(*A*) *Metabolism.* The possible involvement of the metabolism of galactose in the observed hyperpolarization was investigated by using three different approaches:

(*1*) *Nonmetabolizable Analogues.* Figure 1 shows the response of the membrane potential of wild-type cells, in the presence of amytal and DCCD, upon the addition of nonmetabolizable analogues of D-galactose. The analogues chosen, D-fucose and L-sorbose, are attractants (though less powerful than galactose) that work via the galactose receptor (Adler, 1969; Adler et al., 1973). They had been purified prior to use in order to avoid any nonspecific effects of a contaminant. The addition of both analogues caused a transient hyperpolarization (Figure 1), as did the addition of galactose in the same strain and under the same experimental conditions (Eisenbach, 1982; cf. Figure 5 below). Relatively high concentrations of the analogues were used in the experiment shown in Figure 1 because of their lower affinities (compared to galactose) for the galactose receptor (Adler et al., 1973). Also lower concentrations of the analogues were sufficient to induce a hyperpolarization, though smaller than illustrated in Figure 1. The dependence of the magnitude of the hyperpolarization on the analogue D-fucose concentration is shown in Figure 2. Even at the low concentration of 100 μM D-fucose, a hyperpolarization (3.4 mV) was observed.

(*2*) *Mutant Defective in the Metabolism of Galactose.* The strain DF2000 (Fraenkel, 1968), which cannot metabolize galactose, is identical in all other respects with its wild-type parent, AW493. Adler et al. (1973) have demonstrated that strain DF2000 exhibits normal galactose taxis when grown on D-mannose as the sole carbon and energy source. We, therefore, monitored the membrane potential in both AW493 and DF2000 following the addition of D-galactose. Under the conditions used in Figure 1, galactose (1 μM final concen-

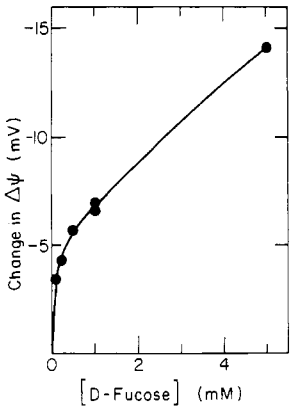

FIGURE 2: Effect of D-fucose concentration on the magnitude of the hyperpolarization stimulated by this chemical. Changes in membrane potential were monitored in strain AW546 by the TPP^+ electrode under the same experimental conditions as in Figure 1. The apparent values of the net changes in membrane potential were calculated as detailed under Experimental Procedures. Each point in the graph was measured with a different sample of bacteria. The average of the apparent membrane potential prior to the addition of D-fucose was 135 ± 12 mV (±SD; three different batches of bacteria were used for this graph).

tration)[2] stimulated similar changes in the apparent values of membrane potential of both AW493 (−4.7 ± 0.3 mV) and DF2000 (−5.0 ± 0.3 mV), when the strains were grown on D-mannose as the sole carbon and energy source. In accordance with the above, similar magnitudes of hyperpolarizations were observed with strains that had been grown on glycerol plus D-fucose (a known inducer for the galactose-binding protein) instead of D-mannose.

(*3*) *Glycolytic Inhibitor.* High concentrations of sodium fluoride (10 mM), a glycolytic inhibitor of *E. coli* (Kaback, 1968; Spring & Wold, 1975), did not prevent the hyperpolarization of AW546 stimulated by galactose (data not shown).

On the basis of these three lines of evidence, it seems that metabolism is not the cause for the hyperpolarization. A partial small contribution of galactose metabolism to the observed hyperpolarization cannot, however, be excluded. A further confirmation of this conclusion will be discussed in conjunction with Figure 3.

(*B*) *Transport.* There are at least six transport systems for galactose in *E. coli* (Silhavy et al., 1978): the methyl β-galactoside (Mgl) transport system, the galactose permease [or galactose-specific transport system (Wilson, 1974a)], the thiomethyl galactoside permease I, the melibiose transport system [or the thiomethyl galactoside permease II (Prestidge & Pardee, 1965; Rotman et al., 1968; Tsuchiya et al., 1977)], the arabinose transport system, and facilitated diffusion by enzyme II for glucose of the phosphoenolpyruvate phosphotransferase system. Among these systems two are known to be electrogenic: the galactose permease (Henderson, 1974) and the melibiose transport system (Tsuchiya et al., 1977; Tokuda & Kaback, 1977). Since only the first two systems, the Mgl and the galactose permease, are inducible by galactose (Wilson, 1974b), our growth conditions (galactose as the sole carbon and energy source) induce only these two systems. We have, therefore, examined them separately for their possible involvement in the hyperpolarization. In the following experiments any marginal effects of the noninduced transport systems would be expected to be equally active in the wild type and its mutant derivatives.

[2] Due to possible interfering processes occurring at high concentrations of galactose, and in order to achieve high specificity, we have intentionally used here a low concentration of galactose. The magnitude of the hyperpolarization was therefore relatively low (cf. Figures 2 and 5).

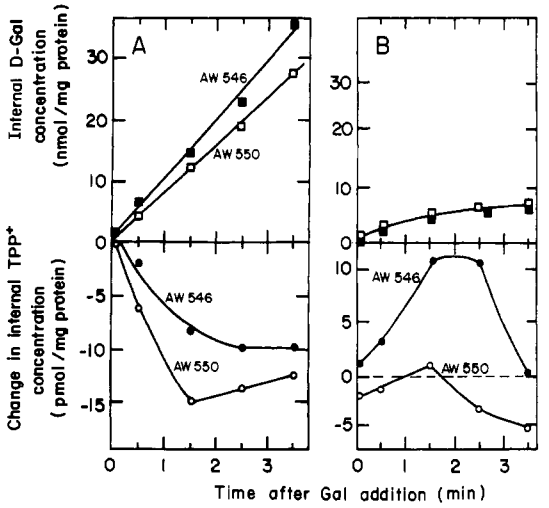

FIGURE 3: Simultaneous measurement of galactose uptake and galactose-stimulated changes in membrane potential. D-[^{14}C]Galactose and [^{3}H]TPP^+ (1 mM and 0.77 μM final concentrations) were used to follow galactose uptake and changes in membrane potential, respectively, by using the filtration technique. [^{3}H]TPP^+Br^- was added to a 2.5-mL suspension of permeabilized bacteria (0.7 mg of protein/mL) followed, when applicable, by the addition of the inhibitors. From this suspension, 100-μL portions were distributed to wide plastic tubes immersed in a constant-temperature bath with rotatory shaking. After 20 min, 2 μL of D-[^{14}C]galactose (0.16 Ci/mol) or, as a control, 2 μL of the suspending medium was added to one of the tubes. The tube was then vigorously shaken in the bath for the specified period of time (up to 5 min), at the end of which 2 mL of 0.1 M LiCl (at room temperature) was added to stop the reaction, and the resulting suspension was filtered through a Uni-Pore polycarbonate membrane (Bio-Rad, 0.6-μm pore diameter). The tube and the membrane were then washed once with 2 mL of 0.1 M LiCl, and the membrane was removed from the apparatus and allowed to dry. Only then was D-galactose or suspending medium added to the next tube to begin the next experimental point. D-Galactose and the suspending medium were used alternately (for n tubes with galactose, n + 1 tubes with suspending medium). When dry, the membrane filters were placed in scintillation vials containing 10 mL of Aquasol (New England Nuclear), and radioactivity was determined by a liquid scintillation counter (Packard Model 3255). Washing the membranes with suspending medium instead of LiCl did not noticeably affect the counts, nor did including 1 μM TPB^-Na^+ in the reaction mixture. The change in internal TPP^+ concentration was obtained by subtracting the base line (counts obtained with additions of suspending medium vs. time after [^{3}H]TPP^+ addition) from the curve obtained with D-galactose addition. Temperature 30 °C. (●, ○) Change in [^{3}H]TPP^+ content of AW546 and AW550, respectively; (■, □) D-galactose content of AW546 and AW550, calculated from the [^{14}C] counts. (A) No inhibitors were present. (B) KCN (5 mM) and DCCD (18 μM) were present. [KCN was used here as the respiratory inhibitor to extend the time length of the hyperpolarization (Eisenbach, 1982).] The hyperpolarization of AW546 in part B corresponds to 16 mV (from −101 to −117 mV).

(*1*) *Galactose Permease.* Since the galactose permease functions by a H^+–galactose symport mechanism (Henderson & Kornberg, 1975; Van Thienen et al., 1977), uptake of galactose via this system should be accompanied by a depolarization and not by a hyperpolarization as was observed above. Figure 3A, which shows the correlation between the uptake of D-[^{14}C]galactose and the simultaneous changes in membrane potential (stimulated by galactose and measured by [^{3}H]-TPP^+), demonstrates that this is indeed the case. Since the apparent K_m of the galactose permease for galactose is of the order of 0.1 mM (Rotman et al., 1968), in this experiment we used a relatively high concentration of galactose (1 mM) to ensure the maximal rate of transport. In the absence of inhibitors, the wild-type strain (AW546) accumulated galactose with a concomitant depolarization (Figure 3A). Under the conditions needed to observe the hyperpolarization, i.e., in the

Table II: Relative Magnitudes of Hyperpolarization, Stimulated by D-Fucose, in Chemotactic *mgl* Mutants[a]

strain	defective gene	rel hyperpolarization[b] (%)	rel transport[c] (%)
OW1	wild-type	100 ± 13	100
OW11	*mglB* (class III)	210 ± 78	9
OW15	*mglA*	122 ± 1	4
OW24	*mglC*	106 ± 14	4

[a] Changes in membrane potential stimulated by purified D-fucose (5 mM final concentration) were monitored by the TPP^+ electrode under the same experimental conditions as in Figure 1. The apparent values of the change in membrane potential were calculated as detailed under Experimental Procedures. The apparent membrane potential prior to the addition of fucose was −102 ± 6 mV (±SD), and the apparent change in OW1 was −35 ± 10 mV. [b] This value (±SD) was calculated from the number of moles of TPP^+ taken up per milligram of protein of the mutant. [c] The values in this column are the relative V_{max} values of galactose transport in the mutants as compared with the V_{max} value of OW1. The numbers of this column are from Ordal & Adler (1974b).

presence of KCN and DCCD, the uptake of galactose was inhibited (cf. Kerwar et al., 1972) and a hyperpolarization was observed (Figure 3B). To confirm that under these conditions the measured galactose transport was mainly through the galactose permease, we repeated this double-labeling experiment in strain AW550, an *mgl* mutant derivative of AW546. As shown in Figure 3, the galactose uptake in the wild-type and *mgl* mutant was almost identical. These observations indicate that the hyperpolarization, stimulated by galactose, is not the consequence of galactose transport via the permease system.

The observation made from the experiment depicted in Figure 3B, i.e., that AW550 had not become hyperpolarized by galactose in spite of accumulating and metabolizing galactose as its parent AW546, further supports the conclusion reached in section A above: the metabolism of galactose is probably not the cause of the hyperpolarization. Galactose was metabolized at the same rate by AW546 and AW550 (not shown).

(*2*) *Mgl Transport System.* Specific mutants in this transport system were used as a means to determine whether or not this system is involved in the hyperpolarization. Many *mgl* mutants are indeed available, but many of them are defective in both transport and chemotaxis to galactose (Ordal & Adler, 1974b). Table II includes the relative magnitudes of the hyperpolarization in *mgl* mutants which were carefully chosen to be defective in transport only. All of the mutants were derived from the wild-type strain OW1, and they differ from it in the specified gene only. As shown in the table, neither one of the mutants lost fucose-stimulated hyperpolarizability, in spite of being practically unable to transport fucose or galactose through the Mgl system. We have chosen to present the results with D-fucose rather than with D-galactose, in order to avoid any contribution to the observed hyperpolarization (even marginal) from the galactose metabolism. [In confirmation of the above results, also galactose was found to stimulate a hyperpolarization in all of these mutants (not shown).] Thus, it seems that the hyperpolarization is not correlated with the Mgl transport system.

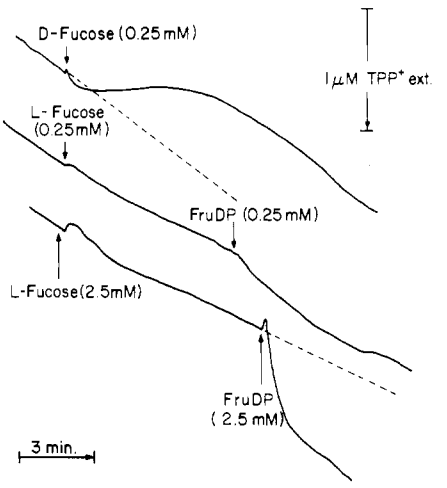

FIGURE 4: Changes in membrane potential stimulated by attractant and nonattractant sugars. The experimental conditions were as in Figure 1, only that 0.5 mg of protein/mL were used and the stimuli were those shown in the figure. Neither L-fucose nor FruDP was purified before use. The apparent membrane potential before the addition of the sugars was −144 ± 4 mV (±SD). The apparent changes in membrane potential were −24 mV for D-fucose, −1 and −3 mV for 0.25 and 2.5 mM L-fucose, and +7 and +23 mV for 0.25 and 2.5 mM FruDP. The dashed lines are extrapolations of the base lines.

(*C*) *Chemotaxis.* If the hyperpolarization is correlated with chemotaxis, it would be expected that a *flaI* mutant will not become hyperpolarized by galactose or D-fucose. The *flaI* is a control gene, the presence of which is required for the expression of all of the genes for motility and chemotaxis (Silverman & Simon, 1974, 1977a; Komeda et al., 1980; Komeda, 1982). A *flaI* mutant is, therefore, a nonflagellated strain which lacks (or has a reduced level of) the proteins (Komeda, 1982) and the known biochemical functions involved in chemotaxis [e.g., MCP methylation (Siverman & Simon, 1977b) and demethylation (Toews et al., 1979), changes in cGMP levels stimulated by attractants or repellents (Black et al., 1980), and the specific binding of serine (Hedblom & Adler, 1980)]. Table III summarizes the relative magnitudes of the hyperpolarizations induced by D-fucose and galactose in *flaI* mutants. In all of these mutants, the hyperpolarization was reduced to 14 ± 2% of that observed for their respective parent strains. Thus, it seems that the hyperpolarization is indeed correlated with chemotaxis. These observations with *flaI* mutants are in accord with those of Szmelcman & Adler (1976) with the reservations previously discussed (Eisenbach, 1982).

This correlation was pursued further by examining the response of wild-type bacteria to nonattractant sugars. [All attractant sugars cause a hyperpolarization (Eisenbach, 1982).] Figure 4 shows the changes in membrane potential stimulated by L-fucose [a metabolizable but nonchemotactic analogue of

Table III: Relative Magnitudes of Hyperpolarization in *flaI* Mutants[a]

parent strain	mutant strain	mutation	stimulus	rel hyperpolarization[b] (%)
HB233	HB261	*flaI*	D-fucose (2.5 mM)	15
AS-1	AS-1*flaI*	*flaI*am88	D-fucose (0.25 mM)	12
RP437	RP3098	Δ(*flaI-flaH*)	D-galactose (1 mM)	14

[a] Membrane potential was followed as in Table II. [b] This value was calculated from the number of moles of TPP^+ taken up per milligram of protein of the mutant. The number of moles taken up by the parent strain of the mutant was considered as 100%.

Table IV: Changes in Membrane Potential of *mglB* Mutants[a]

strain	Gal-binding protein		Gal taxis and transport[b,c]	hyperpolarization by Gal[d]			
	K_m[b]	level[b]		10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
AW546 (wt)	normal	normal	normal	+	+	+	+
AW550	higher	almost normal	defective	−	−	−	−[e]
B275 (wt)	normal	normal	normal	+	+	+	+
AW541	normal	lower	defective	−	−	−	−
AW543	normal	lower	defective	−	−	+	+

[a] Experimental conditions were as in Figure 1. Identical results were obtained with KCN replacing amytal, or in the absence of DCCD. [b] Data taken from Hazelbauer & Adler (1971) and Ordal & Adler (1947b). [c] Capillary assays (Adler, 1973) were performed for all the strains with observations identical with those quoted from the literature in this column. Gal transport means in this table transport through the Mgl system. [d] Plus and minus signs refer to a hyperpolarization or no response, respectively. [e] In a small number of experiments, a small hyperpolarization was observed with 1 mM galactose in this strain.

D-galactose (Adler et al., 1973)] and FruDP [a nonmetabolizable and nonchemotactic sugar (Adler et al., 1973)] in comparison to those stimulated by D-fucose. It is clear that only D-fucose caused a hyperpolarization. Only at very high concentrations of L-fucose was a very small and short hyperpolarization observed, possibly the result of impurities (L-fucose, unlike D-fucose, was not purified before use). FruDP caused a depolarization at both concentrations examined. These observations confirm that the hyperpolarization is correlated to chemotaxis. In other words, it seems that only attractants cause a hyperpolarization under the experimental conditions of this study.

The galactose-binding protein of the Mgl transport system (encoded by the *mglB* gene) also serves as the receptor for galactose taxis (Hazelbauer & Adler, 1971; Ordal & Adler, 1974b). A final test for the correlation of the observed hyperpolarization with chemotaxis would be a strict dependence of the hyperpolarization on the galactose-binding protein. We performed this test in two manners:

(*1*) *Noninducing Conditions.* The galactose-binding protein is an inducible protein. If bacteria are grown in the absence of galactose or its analogue, the protein is not synthesized (Adler, 1969; Boos, 1972). Figure 5A shows that almost no hyperpolarization was observed in AW546 cells that had been grown under noninducing conditions (glycerol replacing galactose as the sole carbon and energy source). AW546 cells that had been grown under inducing conditions (D-fucose present in addition to glycerol) and examined under identical conditions did become hyperpolarized by galactose (Figure 5B).

(*2*) *Mutants Defective in Galactose Binding.* We extended our investigations with *mglB* mutants (cf. Figure 3) to strains with well-defined defects in the binding protein. Table IV includes observations with *mglB* mutants which are defective in the binding of galactose [distinct from *mglB* mutants having other defects (Ordal & Adler, 1974b), which will be discussed elsewhere]. As shown in the table, normal binding of galactose to the galactose-binding protein is, indeed, a prerequisite for the hyperpolarization to occur.

Discussion

This study shows that the galactose-stimulated hyperpolarization is correlated to chemotaxis. Two approaches have been taken here: (1) an indirect approach eliminating other alternatives, besides chemotaxis, and (2) a direct approach showing correlation with chemotaxis.

The evidence from the indirect approach is summarized as follows: (a) The hyperpolarization is not the consequence of galactose metabolism. Nonmetabolizable analogues of galactose also stimulated a hyperpolarization (Figure 1). A mutant defective in galactose metabolism became hyperpolarized (section A2 under Results). A hyperpolarization was not observed in mutants which normally metabolize galactose (Figure 3 and Table IV), and the hyperpolarization was not inhibited by a glycolytic inhibitor (section A3 under Results). (b) The hyperpolarization is not the consequence of galactose transport. All of the noninducible transport systems do not participate in the hyperpolarization since the hyperpolarization *is* inducible (Figure 5). The two remaining transport systems which are inducible by galactose are also not related to the hyperpolarization. A hyperpolarization is observed at galactose concentrations 100-fold lower than the K_d of the galactose permease (Figure 5). Furthermore, inhibiting the galactose permease did not prevent the hyperpolarization, rather it facilitated the observation of the hyperpolarization (Figure 3). A hyperpolarization was observed in *mgl* mutants which do not transport galactose through the Mgl transport system but carry normal galactose taxis (Table II). (c) The hyperpolarization is not stimulated by nonattractant sugars (Figure 4). (d) The hyperpolarization is strictly dependent on the galactose-binding protein (Figure 5 and Table IV).

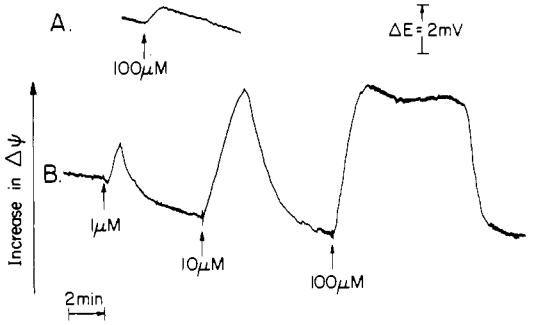

FIGURE 5: Requirement for inducing growth conditions. The experimental conditions were as in Figure 1, only that 0.5 mg of protein/mL was used. D-Galactose was added where indicated in aliquots of 3 μL from stock solutions at various concentrations to yield the final concentrations indicated in the figure. (A) Bacteria that were grown with glycerol (55 mM) as the sole carbon and energy source. (B) As in (A), only that the growth medium contained also D-fucose (10 mM).

The direct evidence for a correlation between hyperpolarization and chemotaxis is that *flaI* mutants, in which the whole chemotaxis machinery is repressed, had only 12–15% of the hyperpolarization exhibited by their respective chemotactic parents (Table III).

This study does not suggest an explanation for the way in which the hyperpolarization is correlated to chemotaxis. This will be the topic of a subsequent communication in which a comparison between various generally nonchemotactic mutants (*che* mutants), *trg* mutants, and paralyzed mutants (*mot* mutants) will be described (M. Eisenbach and T. Raz, unpublished results). At this point, however, we may suggest

that the hyperpolarization is not correlated with the excitation process [the process of transducing the primary signal between the chemoreceptors and the flagella (Springer et al., 1979)] because the latter is too fast [≤0.2 s (Segall et al., 1982)] to account for the much slower hyperpolarization. Furthermore, since the hyperpolarization cannot be observed in most strains examined in the absence of inhibitors (Eisenbach, 1982; Figure 3), it is probably the reflection of another process, possibly an ion flux, that is related to chemotaxis. This topic and the generality of this phenomenon for other attractants and repellents are under extensive investigation.

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Registry No. D-Galactose, 59-23-4; galactose permease, 39279-39-5; D-fucose, 3615-37-0; L-sorbose, 87-79-6.

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