Behavioral Responses of *Escherichia coli* to Changes in Temperature Caused by Electric Shock†

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The behavioral response of *Escherichia coli* to electric shock in 10^{-2} M potassium phosphate plus 10^{-4} M potassium EDTA was studied. When presented with a 150-V/cm electric shock that lasted 250 ms, the bacteria at first exclusively ran, then exclusively tumbled, and finally returned to their original running and tumbling. This response is due to increased temperature caused by the electric shock, i.e., to thermotaxis, and it is mediated by the chemotaxis machinery. A more severe electric shock, 150 V/cm for 550 ms, caused cells to tumble immediately, and then they went back to their original running and tumbling. The mechanism of that response is unknown since, unlike known thermotaxis, it does not require the chemotaxis machinery.

Eukaryotic organisms can be attracted by small electric fields, but they behave negatively in response to electric shock (for examples, see references 8, 16, 23, 28, and 34). It is not understood exactly how this is brought about, but effects of electricity are known to be related to cell membrane potential and ion channel activities (13).

Relatively little work has been done on the behavioral response of bacteria to electricity (3, 10, 11, 24). We previously used a continuous low voltage, 3 V/cm, and found that *Escherichia coli* and *Salmonella typhimurium* migrated toward one pole or the other (3).

For the present study, we used short bursts of high voltage, 150 V/cm, to investigate the behavioral responses of *E. coli*. For a 250-ms duration of such an electrical shock, the bacteria first ran and then tumbled; the temperature increased, so we believe that the bacteria were carrying out thermotaxis and that, as expected for thermotaxis (14), the chemotaxis machinery was required. For 150-V/cm shocks lasting 550 ms, the bacteria only tumbled; the temperature increased even further, but the chemotaxis machinery was not required for this response.

MATERIALS AND METHODS

Bacterial strains and media. The *E. coli* strains used here are described in Table 1. Bacteria were grown in tryptone broth (1% Bacto Tryptone, 0.5% NaCl) to an optical density at 590 nm of 0.3 to 0.4. They were then washed twice with 10^{-2} M potassium phosphate buffer (pH 7.0)– 10^{-4} M potassium EDTA (motility medium) (1) and finally resuspended in that medium at an optical density at 590 nm of 0.1 (about 7×10^7 cells per ml). Methionine auxotrophs were used with 0.1 mM L-methionine present, since it is required for tumbling (1, 33).

Apparatus for study of bacterial response to electric shock. The apparatus for observing bacterial responses to high-voltage electric shock is diagrammed in Fig. 1. An 11-cm-long chamber was built from leucite plastic, except for a

glass window in the middle compartment. Bacterial behavior was observed through the microscope and recorded on videotape for study by a computerized motion analysis system (29). Bacteria in motility medium (0.5 ml) were put into the middle glass-bottomed compartment (2 cm by 2.5 cm by 1 mm) and covered with a glass coverslip. At each end of this compartment was a compartment (4.5 cm by 2.5 cm by 8 mm) containing 9 ml of 1% agarose (electrophoresis grade; Bethesda Research Laboratories) in motility medium. These two end compartments of the chamber served as salt bridges to prevent by-products of electrochemical reactions around the electrodes from reaching the bacteria. Each of these two compartments contained, as far away from the bacteria as possible, a platinum electrode attached to a power supply. For consistent results, a fresh chamber was used for each experiment; however, it was possible to repeat at least six experiments in the same chamber without big changes in results.

A power supply was built (Instrumentation Systems Center, University of Wisconsin-Madison) which can deliver 1,000-V electric pulses for 1 ms to 2 s. A tone maker and timer controlled the power supply (Fig. 1); once the control button was pushed, it generated a tone sound, and an electric pulse was delivered 20 s after the tone. The electric field was monitored by an oscilloscope (not shown in Fig. 1). An electronic thermoprobe (model DP30; Omega Technologies Co.) was embedded into the bacterial compartment to monitor the change in temperature (not shown in Fig. 1).

The electric field for the bacterial compartment was determined with a voltage-ohm meter to be 150 V/cm, and we shall refer to it as that in the text. In theory, however, it is 320 V/cm: the two agarose blocks, taken together, are 4.5 times longer and 8 times thicker than the bacterial compartment, so their combined resistance should be about $4.5/8 = 0.56$ that of the bacterial compartment; thus $1/(1 + 0.56) = 0.64$ of the voltage drop should be across the bacterial compartment; $640 \text{ V}/2 \text{ cm} = 320 \text{ V/cm}$. An electric field of 150 V/cm, being $15 \text{ mV}/\mu\text{m}$, would change the membrane of a cell $1 \mu\text{m}$ in diameter by 7.5 mV at either side of the cell.

RESULTS

Unstimulated wild-type *E. coli* cells both run and tumble; in response to attractants they run, and in response to

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TABLE 1. Response to electric shock by *E. coli* chemotaxis mutants

Strain	Reference or source	Genotype	Response to:	
			Type I shock ^a	Type II shock ^b
AW405	21	Chemotactically wild type	+	+
AW518	12	<i>tsr</i> (MCP I ⁻)	±	+
AW539	21	<i>tar</i> (MCP II ⁻)	±	+
AW701	17	<i>trg</i> (MCP III ⁻)	+	+
RP3525	32	<i>tap</i> (MCP IV ⁻)	+	+
CP362	26	MCP I ⁻ II ⁻ III ⁻ IV ⁻	-	+
AW990	This study	CP362/ <i>ptsr</i> ⁺ (pCS5) ^c	+	+
AW991	This study	CP362/ <i>ptar</i> ⁺ (pNT201) ^c	+	+
AW992	This study	CP362/ <i>ptrg</i> ⁺ (pGB1) ^c	-	+
AW993	This study	CP362/ <i>ptap</i> ⁺ (pVB8) ^c	-	+
RP1254	J. S. Parkinson	<i>ΔcheR</i>	-	+
RP4971	J. S. Parkinson	<i>ΔcheB</i>	-	+
RP1788	19	<i>ΔcheA</i>	-	+
RP1078	19	<i>ΔcheW</i>	-	+
RP5232	J. S. Parkinson	<i>ΔcheY</i>	-	+
RP1616	J. S. Parkinson	<i>ΔcheZ</i>	±	+
HCB326	35	Missing 4 MCPs and 6 Che proteins	-	+

^a *E. coli* responded to a 150-V/cm, 250-ms electric shock by exclusively running for about 15 s and then primarily tumbling for about 25 to 30 s before they go back to running and tumbling. + means that such a response was observed; - means that no response was observed; ± means that such a response was observed but the time for exclusively running and tumbling was less than that of the wild type. The *ΔcheZ* mutant responded to the shock with 10 s of running and then returned to the original tumbling.

^b *E. coli* cells respond to a 150-V/cm, 550-ms electric shock by exclusively tumbling immediately for about 10 s, then becoming primarily tumbling for another 20 to 25 s, and then returning to their preshock behavior. + means that such a tumbling response was observed. The *cheB* and *cheZ* cells, both tumbling mutants, became even tumblers after the type II electric shock.

^c Plasmids used were pCS5 (from F. W. Dahlquist), pVB8 (25), pNT201 (6), and pGB1 (7).

repellents they tumble (2, 9). Such bacterial responses can be studied by a computerized motion analysis system: running causes increased linear speed and decreased angular speed, whereas tumbling causes decreased linear speed and increased angular speed (29).

Effects of electric shock on bacterial behavior. We used the apparatus shown in Fig. 1 to administer an electric shock of 1,000 V across the 11-cm-long chamber. The duration of the electric shock varied; we catalog the shock as type I (typically 250 ms, but less than 450 ms), type II (typically 550 ms, but 450 to 750 ms), type III (750 ms to 1 s), and type IV (more than 1 s).

FIG. 1. Apparatus for observing bacterial response to high-voltage electric shock. Bacteria and 1% agarose were in motility medium. The power supply, built locally, can deliver 1,000-V electric pulses for 1 ms to 2 s. See Materials and Methods for details.

Figure 2 shows the response of wild-type *E. coli* AW405 cells to a type I electric shock. When a 150-V/cm electric pulse (1,000 V over the entire chamber) lasting 250 ms was delivered, the bacteria exclusively ran for about 15 s (fast initially, then slower) and then became primarily tumbling for another 25 to 30 s before returning to the original running and tumbling behavior. The type I electric shock had almost no effect on the viability of the cells (see Fig. 5).

Figure 3 shows the response of wild-type *E. coli* AW405 cells to a type II electric shock. A 150-V/cm electric pulse lasting 550 ms did not give any running response; instead the cells were exclusively tumbling for about the first 10 s and then became primarily tumbling for another 20 to 25 s before returning to the original running and tumbling behavior. The viability of the cells was not affected much by the type II electric shock (see Fig. 5).

When the 150-V/cm electric shock lasted between 750 ms and 1 s (type III), the cells lost their motility immediately but recovered it in about 6 min (Fig. 4). Most of the cells were still viable after this type III electric shock (Fig. 5). A 150-V/cm electric pulse lasting longer than 1 s (a type IV electric shock) caused long-lasting loss of motility (Fig. 4) and death (Fig. 5).

The response to the electric shock is related to the ionic concentration of the bacterial suspension. Compared with the bacteria in motility medium, bacteria in medium with a higher ionic concentration needed an electric shock of shorter duration or lower voltage to bring about the same response. In contrast, when the ionic concentration was diluted 10-fold the response was eliminated (data not shown).

The supernatant solution of cells shocked for 0, 250, 550, 900, or 1,500 ms in motility medium was collected to test whether there was any attractant or repellent produced by electric shock. Use of the capillary assay (1) revealed no attractant or repellent activity in the supernatant solutions

FIG. 2. Response of wild-type *E. coli* AW405 to type I electric shock (150 V/cm, 250 ms) delivered after 20 s, and the consequent change in temperature. The top panel represents the behavioral response to electric shock as observed in the microscope and judged subjectively. +++, running and tumbling; —, running exclusively; ■■■, tumbling exclusively or primarily. The two middle panels show behavioral changes in response to the electric shock as studied by the computerized motion analysis system (29). This technique measures both the average linear speed, which is the speed of movement in a straight line in micrometers per second; and the average angular speed, which is the rate of change in direction of movement in degrees per video image frame. Increased linear speed and decreased angular speed indicate running; decreased linear speed and increased angular speed indicate tumbling. As an example, wild-type AW405 in an unstimulated situation had a linear speed of 15 $\mu\text{m}/\text{s}$ and an angular speed of 44° per frame. When the attractant 10^{-2} M L-serine was added, the linear speed increased to 19 $\mu\text{m}/\text{s}$ and the angular speed decreased to 27° per frame. When the repellent 10^{-1} M L-leucine was added to the cells, the linear speed decreased to 10 $\mu\text{m}/\text{s}$ and the angular speed increased to 71° per frame. The bottom panel shows changes in temperature on administration of the electric shock; the temperature was measured by a thermosensitive probe embedded into the bacterial compartment.

(data not shown). This result was confirmed by the temporal assay (20), in which supernatant solutions of shocked cells did not cause wild-type cells to exclusively run or primarily tumble. Therefore it seems that response to electric shock is not due to the response to chemicals excreted during the electric shock.

FIG. 3. Response of wild-type *E. coli* AW405 to type II electric shock (150 V/cm, 550 ms) delivered after 20 s, and the consequent change in temperature. See the legend to Fig. 2 and Materials and Methods for detailed information.

Relationship between responses to electric and chemical stimuli. Is the response to electric shock additive to the chemotaxis response? Addition of 10^{-1} M L-leucine during the exclusively running period in response to a 150-V/cm 250-ms electric shock made cells tumble immediately, and addition of 10^{-2} M L-serine during the tumbling period in response to the same electric shock made cells run at once (data not shown). Addition of 10^{-2} M L-serine during the tumbling period in response to a 150-V/cm 550-ms electric shock made the cells run at once (data not shown). Thus the effect of electric shock can be overcome by chemical stimuli, at least at the concentrations of chemicals used here.

To further study the relationship between responses to electric and chemical stimuli, we tested known chemotaxis mutants missing chemotaxis receptors or Che proteins to see how they respond to electric shock (Table 1). The major chemoreceptors of *E. coli* are methyl-accepting chemotaxis proteins (MCPs) I and II, called Tsr and Tar, respectively, whereas MCP III and IV, called Trg and Tap, respectively, are relatively less abundant. For response to the type I electric shock, mutants with mutations in *tsr* (AW518) or *tar*

FIG. 4. Effects of type III (150 V/cm, 900 ms) and type IV (150 V/cm, 1,500 ms) electric shock on motility of bacteria. Motility of *E. coli* AW405 was observed through a microscope and recorded on videotape. The percentage of motile cells was estimated by analyzing the videotape.

(AW539) showed reduced responses, i.e., they exclusively ran for less time (about 8 s) and then exclusively tumbled for less time (about 10 s) than did the wild type (Table 1; also see the example of AW518 without L-aspartate in Fig. 6). Strains with mutations in *trg* (AW701) and *tap* (RP3525) had responses to the type I electric shock similar to that of the wild type (Table 1). A strain missing all the known MCPs (CP362) had no response to the type I electric shock (Table 1). However, CP362 transformed with plasmids carrying *tsr* or *tar* did respond to the type I electric shock (Table 1); in contrast, CP362 with plasmids carrying *trg* or *tap* did not

FIG. 5. Effects of electric shock on viability of bacteria. Wild-type *E. coli* AW405 was subjected to a 150-V/cm electric shock for different times as shown on the x axis. The shocked cells were diluted and plated, and colonies on the plates were counted.

respond to the type I electric shock (Table 1; for details about *trg*, see below). Strains with *cheA*, *cheY*, *cheW*, *cheB*, or *cheR* mutations did not respond to the type I electric shock (Table 1). A strain with a *cheZ* mutation (RP1616) showed a reduced response to the type I electric shock: cells ran but only for 10 s after the shock before they went back to tumbling. (A shortened response of *cheZ* mutants to chemical stimuli has been reported [30]). A mutant missing all the chemotaxis receptors and Che proteins (HCB326) showed no response to the type I electric shock (Table 1). Therefore it is clear that the chemotaxis machinery is crucial for response to the type I electric shock.

All the strains described above showed tumbling responses to the type II electric shock. Strains with mutation in MCPs had similar responses to the wild type; for *cheA*, *cheY*, *cheW*, and *cheR* mutants, which are smooth, a 150-V/cm electric shock lasting 550 ms caused tumbling for 10 to 15 s before the bacteria became exclusively smooth again; for *cheB* and *cheZ* strains, which are tumbling already, the type II electric shock made them tumblier; strain HCB326 (missing all the MCPs and Che proteins) also showed tumbliness after a type II electric shock (Table 1). Therefore, in contrast to the case for the type I electric shock, the type II electric shock can make cells tumble without any chemotaxis receptors or Che proteins.

We found from tethered-cell experiments that cells subjected to type I electric shock rotate their flagella clockwise in correspondence to the tumble response, whereas cells subjected to type II electric shock rotate their flagella counterclockwise in correspondence to the tumble response (data not shown).

Just as for the wild type, a 150-V/cm electric shock lasting 900 ms made all the above mutants nonmotile, and, similar to the wild type, motility was recovered after a time (2 to 7 min). Just as for the wild type, a 150-V/cm electric shock lasting 1,500 ms had lethal effects on all of the mutants named above.

Relationship between response to electric shock and thermotaxis. Thermotaxis is the behavioral response to temperature changes. In *E. coli*, increasing the temperature results in running and decreasing the temperature causes tumbling (14). In motility medium, electric shock would heat the cell solution, so responses to electric shock may well be expected to be due to thermotaxis.

The following evidence suggests that response to the type I electric shock (150 V/cm, 250 ms) is indeed related to thermotaxis. (i) We embedded an electronic thermoprobe into the bacterial compartment to monitor the temperature changes due to electric shock. Figure 2 shows that type I electric shock did cause an increase in temperature in the bacterial chamber. (ii) The results listed in Table 1 for the type I electric shock parallel the data for thermotaxis (14). (iii) The *tsr* mutant (AW518) gives an inverted response to temperature change in the presence of 10^{-3} M L-aspartate (exclusively tumbling instead of exclusively running in response to increased temperature) (22). We have tested the *tsr* mutant (AW518) in response to 250 ms of 150-V/cm (type I) electric shock in the presence of 10^{-3} M L-aspartate and found that it showed an inverted response to the electric shock too (exclusively tumbling first, then exclusively running) (Fig. 6). (iv) It is known that chemical attractants make *cheB* mutants run (although at higher than normal concentrations) (27), but thermotaxis fails to do so (15). Here we found that the type I electric shock is also not able to make a *cheB* mutant run (Table 1). (v) Unlike chemotaxis, no specific MCP is required for thermotaxis (14); this also

FIG. 6. Responses of a *tsr* mutant (AW518) to type I electric shock (150 V/cm, 250 ms) delivered after 20 s, with or without the presence of 10^{-3} M L-aspartic acid. See the legend to Fig. 2 and Materials and Methods for detailed information. Symbols: ●, AW518 in motility medium; ○, AW518 in motility medium plus 10^{-3} M L-aspartate.

appears true for the response to the type I electric shock, for which either MCP I or MCP II was able to mediate a response (Table 1). (vi) There is no behavioral change on thermal stimulation for strain CP362 carrying a *trg* plasmid, but when this strain is mixed with 1.6×10^{-3} M phenol, it runs in response to increased temperature (25). Here we report that the type I electric shock caused no behavioral change of AW992 (CP362/*ptrg*⁺) (Table 1) but did make this strain respond when it was mixed with 1.6×10^{-3} M phenol (data not shown). Thus, even low-copy-number receptors can mediate the response to electric shock, as they can to temperature.

The type II electric shock causes an even more severe transient increase of temperature in the chamber (Fig. 3), but the tumbling response to such shock is not related to known thermotaxis since this tumbling is independent of the chemotaxis machinery (Table 1) whereas the known thermotaxis requires MCP and Che proteins (14). However, it seems most likely that this is a novel kind of response to

especially high temperature; the magnitude of the jump is so great that the chemotaxis machinery is bypassed.

The type III or type IV electric shocks generated a 43 and 57°C increase, respectively (data not shown). It was not surprising that such large increases in temperature would make cells nonmotile or nonviable.

DISCUSSION

In this paper we report that *E. coli* cells exhibit behavioral responses to high-voltage electric shock. A 150-V/cm electric shock (1,000 V over the chamber) lasting 250 ms (type I electric shock) makes wild-type *E. coli* cells first run and then tumble before they go back to running and tumbling (Fig. 2). A 150-V/cm electric shock lasting 550 ms (type II electric shock) makes wild-type *E. coli* cells tumble immediately and then return to running and tumbling (Fig. 3). A 150-V/cm electric shock for 900 ms makes cells nonmotile immediately, and then the motility of the cells gradually recovers (Fig. 4). A 150-V/cm electric shock for 1,500 ms causes irreversible damage to the bacteria (Fig. 5).

We believe that heat generated by the type I electric shock (a change of 15°C [Fig. 2]) causes the behavioral response. In other words, we believe that we are dealing here with thermotaxis. When such an electric shock is delivered, the abrupt temperature jump most probably saturates the response mechanism and the cells continue to run for about 20 s, while the environment has already started cooling. Such behavior is consistent with the response to chemical attractants (see Fig. 3 of reference 5). It is clear that the type I electric shock operates at least via MCP I, II, or III. Thermotaxis also uses MCP I, II, or III and in addition has been shown to use MCP IV (25).

During the type II electric shock there is an even greater transient increase of temperature, a change of 35°C (Fig. 3), than during the type I shock. There is no report that such a high temperature can make cells tumble, and to our knowledge no other device has been used to raise the temperature this steeply. This response is not ordinary thermotaxis, since no chemotaxis machinery is required. However, it seems likely that it is a new form of response to high temperature, i.e., a new form of thermotaxis.

It is not known what causes the tumbling behavior in response to type II electric shock. This tumbling response to type II electric shock looks like a repellent response, but it does not require any MCP or Che proteins (Table 1), unlike known thermotaxis (14). It is interesting that flagella undergo counterclockwise rotation during this tumbling period (see above), whereas ordinary tumbling results from clockwise rotation (4, 31). The shock could physically disrupt the flagellar bundle and make the cell tumble. The tumbling response to type II electric shock is similar to the tumbling response to high osmolarity reported before, which also involves counterclockwise rotation of the flagella and bypasses the chemotaxis system (17a, 18). Both responses could serve as an emergency protection for cells to run away from strong negative stimuli without first having to go through the sensory system.

The arrest caused by type III and type IV electric shocks must be caused by the resulting high temperature jumps.

Electric shock is a method which can generate a stimulus quickly, and it can be easily turned on and off. The intensity of an electric shock can be controlled precisely. Thus electric shock supplies a convenient way to study some questions about behavior. A disadvantage for electric shock as used here is that it acts simply by generating heat. At a

minimum, electric shock can be used to provide a thermal stimulus more rapidly and more briefly than the ordinary thermotaxis method does (14). Beyond that, electric shock can reveal currently unknown behavior, which is the case for response to the type II electric shock. (There are other ways of heating cells rapidly, e.g., with a laser.)

Finally, there is promise for using electric shock as a novel stimulus. If the ionic strength of the medium is reduced to a level at least 10 times lower than that used here, or if effective heat removal is provided, thermotaxis will no longer be significant, yet the electric field strength will remain the same. Without involvement of heat, and as a function of field strength, one could then study the biological effects of electric shock directly.

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