Effects of Organic Antagonists of Ca\textsuperscript{2+}, Na\textsuperscript{+}, and K\textsuperscript{+} on Chemotaxis and Motility of Escherichia coli

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Various Ca\textsuperscript{2+} antagonists used in animal research, many of them known to be Ca\textsuperscript{2+} channel blockers, inhibited Escherichia coli chemotaxis (measured as entry of cells into a capillary containing attractant). The most effective of these, acting in the nanomolar range, was ω-conotoxin GVIA. The next most effective were gallopamil and verapamil. At concentrations around 100-fold higher than that needed for inhibition of chemotaxis, each of these antagonists inhibited motility (measured as entry of cells into a capillary lacking attractant). Various other Ca\textsuperscript{2+} antagonists were less effective, though chemotaxis was almost always more sensitive to inhibition than was motility. Cells treated with each of these Ca\textsuperscript{2+} antagonists swam with a running bias, i.e., tumbling was inhibited. Similarly, some Na\textsuperscript{+} antagonists used in animal research inhibited bacterial chemotaxis. E. coli chemotaxis was inhibited by saxitoxin at concentrations above 10\textsuperscript{-7} M, while more than 10\textsuperscript{-4} M was needed to inhibit motility. Cells treated with saxitoxin swam with a tumbling bias. In the case of other Na\textsuperscript{+} antagonists in animals, aconitine inhibited bacterial chemotaxis 10 times more effectively than it inhibited motility, and two others inhibited chemotaxis and motility at about the same concentration. In the case of K\textsuperscript{+} antagonists used in animal research, 4-aminopyridine blocked E. coli chemotaxis between 10\textsuperscript{-3} M and, totally, 10\textsuperscript{-2} M, while motility was not affected at 10\textsuperscript{-2} M; on the other hand, tetraethylammonium chloride failed to inhibit either chemotaxis or motility at 10\textsuperscript{-2} M.

Ion channels are membrane components which function to allow rapid entry or exit of ions into or out of cells. In eukaryotic cells, a wide variety of pharmacological agents are known to block ion channel proteins (23). Several of these agents have been shown to inhibit specifically and to bind with high affinity to either Ca\textsuperscript{2+}, Na\textsuperscript{+}, K\textsuperscript{+}, or Cl\textsuperscript{−} ion channels, while in other cases the inhibition is not specific (23). Besides their use in the characterization of ion channels, organic channel blockers have been employed to isolate and purify channel proteins, such as the acetylcholine receptor (34) and the voltage-gated Na\textsuperscript{+} channel (10, 11).

Ion channels have been demonstrated also in bacteria (for a review, see reference 5). Ion channels have been located in both the cytoplasmic (inner) and outer membranes of gram-negative bacteria such as Escherichia coli. Porphyrin, the first class of bacterial ion channels to be discovered, are located in the outer membrane of gram-negative bacteria and function as molecular sieves to allow passage of small compounds (41). Mechanosensitive channels from the cytoplasmic membrane of E. coli and other species have been studied (4, 12, 14, 15, 22, 28, 31, 54, 55, 66). K\textsuperscript{+} transporters and K\textsuperscript{+} channels are known to occur in the cytoplasmic membrane of E. coli (14, 25, 38, 66). A K\textsuperscript{+} channel occurs in Streptomyces lividans (17, 29, 49), and a glutamate receptor which is a K\textsuperscript{+} channel has been discovered in cyanobacteria (13). The K\textsuperscript{+} channels found in bacteria closely resemble those of animals (17, 25, 29, 38, 49, 67). Ca\textsuperscript{2+} channels have been reported for the cytoplasmic membrane of E. coli (16) and Bacillus subtilis (33). The Ca\textsuperscript{2+} channel of E. coli consists of inorganic polyphosphate bound together with poly-β-hydroxybutyrate (16); it appears that animal-like Ca\textsuperscript{2+} channels are not coded for by the E. coli genome (3). Na\textsuperscript{+} channels have not been reported for bacteria to our knowledge and appear not to be coded for by the E. coli genome (3).

Inorganic cations play a role in bacterial taxis. (For reviews of chemotaxis, see references 19 and 51.) In B. subtilis, Ca\textsuperscript{2+} has been shown to be involved in chemotaxis (44). Several organic Ca\textsuperscript{2+} channel blockers in animals have been reported to inhibit chemotaxis of B. subtilis: ω-conotoxin, verapamil, nitrendipine, and diltiazem (32). In Spirochaeta aurantia, chemotaxis was blocked by inhibitors affecting animal Ca\textsuperscript{2+} channels (botulinum toxin A), Na\textsuperscript{+} channels (aconitine, tetrodoxin, and sea anemone venom), and K\textsuperscript{+} channels (scorpion venom and tetraethylammonium) (20). In Spirillum volutans various inorganic and organic agents, some of them neurotoxic, produce uncoordination of flagella (9, 27). Ca\textsuperscript{2+} produces constant tumbling of E. coli in the presence of a Ca\textsuperscript{2+} ionophore (43). (See also the discussion about Ca\textsuperscript{2+} and E. coli taxis in the paper by Brey and Rosen [7].) Ca\textsuperscript{2+} has been implicated in photophobias responses of Phoroidium uncinitum, a cyanobacterium (or blue-green alga), by the use of ruthenium red, lanthanum, and a calcium-conducting ionophore (21, 39, 40). Ca\textsuperscript{2+} is also involved in the gliding motility of myxobacteria (65). Ca\textsuperscript{2+} plays a role in taxis by Halobacterium salinarum, an archaeon (2, 39).

More recent work documents further that Ca\textsuperscript{2+} is involved in E. coli chemotaxis (56–59, 62), but how the Ca\textsuperscript{2+} acts remains to be determined. The Ca\textsuperscript{2+} antagonist ω-conotoxin inhibits E. coli chemotaxis (59). We (56, 58) and Watkins et al. (62) have reported that the cytoplasmic concentration of Ca\textsuperscript{2+} rises when bacteria encounter repellents (which make them tumble) and falls with attractants (which make them run). In addition, mutants having a high concentration of Ca\textsuperscript{2+} are tumbling (57). (For recent reviews on the role of calcium ions in...
bacteria, including bacterial chemotaxis, see references 24, 42, and 52.

This communication reports an investigation on the effect of organic Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\) antagonists on E. coli chemotaxis and motility by E. coli. The aim of this survey is to identify ions involved in E. coli chemotaxis and to find pharmacological agents which could potentially be used to characterize, and ultimately to purify, ion channel components or other ion-binding components involved in the chemotaxis mechanism.

### MATERIALS AND METHODS

**Bacterial strains.** AW405 (36), AW574 (60), and RP487 (45) were used as chemotactically wild-type strains of E. coli.

**Growth conditions.** Cells were grown in tryptone broth and then in Vogel-Bonner medium (63) containing 50 mM glycerol (except that 25 mM in-lactate was used for the experiment for Fig. 1) plus the required amino acids at 1 mM.

**Chemotaxis assay.** Cells were grown in the Vogel-Bonner growth medium by shaking at 35°C to an optical density of 0.4 to 0.6 at 590 nm. Then they were harvested by centrifugation at 6,000 \( \times g \) for 3 min. The supernatant fluid was discarded, the pellet was resuspended, and chemotaxis medium was added (10 mM K\(^{+}\) phosphate [pH 7.0], 0.1 mM K\(^{+}\) EDTA, and, for RP487, 0.1 mM l-methionine). This was followed by two more such washes in chemotaxis medium and, finally, the cells were resuspended in chemotaxis medium to an optical density of 0.005 at 590 nm (about 4 \( \times \) 10\(^7\) bacteria/mL). Chemotaxis was assayed in chemotaxis medium by the capillary method (1) for 3 min. The supernatant fluid was discarded, the pellet was resuspended, and chemotaxis medium was added.

### RESULTS

We have tested the effects of a number of organic ion antagonists known to be active in animals on E. coli chemotaxis and motility. By use of the capillary assay, the dose required to inhibit chemotaxis by 50% was measured for each antagonist tested and the dose required to inhibit motility by 50% was also indicated at 30°C. α-Serine (1 mM) or l-aspartate (10 mM) was used as an attractant in the capillary tubes (36). The cells in the capillary were plated on tryptone broth agar, and colonies were counted after incubation at 37°C overnight. Motility was assayed in the same way, but attractant was omitted. Antagonists to be tested for their effects on chemotaxis and motility were added to both the cell suspension and the capillary at equal concentrations to eliminate a gradient of the antagonists. Each experiment was repeated several times, and typical results are presented.

### Table 1. Drug concentrations required to inhibit E. coli chemotaxis, motility, and survival

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration producing 50% inhibition of:</th>
<th>Ratio of concn producing 50% inhibition of motility/concn producing 50% inhibition of chemotaxis</th>
<th>Concentration producing 50% loss of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium antagonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Conotoxin GVIA(^{b,c})</td>
<td>0.2 µM (0.01 µM)</td>
<td>13 (100)</td>
<td>No effect at 1 µM</td>
</tr>
<tr>
<td>Gallopamil(^{d})</td>
<td>5 µM</td>
<td>100</td>
<td>No effect at 1 mM</td>
</tr>
<tr>
<td>Verapamil(^{d})</td>
<td>10 µM</td>
<td>65</td>
<td>No effect at 1 mM</td>
</tr>
<tr>
<td>Diltiazem(^{d})</td>
<td>50 µM</td>
<td>16</td>
<td>No effect at 1 mM</td>
</tr>
<tr>
<td>Tiapamil(^{d})</td>
<td>50 µM</td>
<td>10</td>
<td>50 µM</td>
</tr>
<tr>
<td>Chlorpromazine(^{d})</td>
<td>1 µM</td>
<td>10</td>
<td>70 µM</td>
</tr>
<tr>
<td>Tetraamine(^{d})</td>
<td>10 µM</td>
<td>10</td>
<td>50 µM</td>
</tr>
<tr>
<td>Nifedipine(^{e})</td>
<td>30 µM</td>
<td>10</td>
<td>5 mM</td>
</tr>
<tr>
<td>Nisoldipine(^{e})</td>
<td>80 µM</td>
<td>4</td>
<td>5 mM</td>
</tr>
<tr>
<td>Dibucaine(^{e})</td>
<td>37 µM</td>
<td>3</td>
<td>50 µM</td>
</tr>
<tr>
<td>Nitrendipine(^{e})</td>
<td>100 µM</td>
<td>3</td>
<td>5 mM</td>
</tr>
<tr>
<td>Prenylamine(^{e})</td>
<td>7 µM</td>
<td>2</td>
<td>1 mM</td>
</tr>
<tr>
<td>Bepridil(^{e})</td>
<td>5 µM</td>
<td>1</td>
<td>100 µM</td>
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<td>Sodium antagonists</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Saxitoxin(^{e})</td>
<td>10 µM</td>
<td>100</td>
<td>No effect at 1 mM</td>
</tr>
<tr>
<td>Aconitine(^{e})</td>
<td>10 µM</td>
<td>10</td>
<td>No effect at 1 mM</td>
</tr>
<tr>
<td>Lidocaine(^{e})</td>
<td>10 µM</td>
<td>1</td>
<td>1 mM</td>
</tr>
<tr>
<td>Procaine(^{e})</td>
<td>5 µM</td>
<td>1</td>
<td>50 mM</td>
</tr>
<tr>
<td>Potassium antagonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminopyridine(^{g})</td>
<td>2 mM</td>
<td>5</td>
<td>No effect at 10 mM</td>
</tr>
<tr>
<td>Tetraethylammonium chloride(^{g})</td>
<td>&gt;10 mM</td>
<td>&gt;10 mM</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

\(^{a}\) Both chemotaxis and motility were measured by the capillary assay in chemotaxis medium as described in Materials and Methods. Bacteria were treated as described in Materials and Methods. Cells were incubated for 30 min at 30°C in chemotaxis medium with different concentrations of each antagonist and were then presented with capillaries containing the antagonist and 10 mM l-aspartate or no attractant, all in chemotaxis medium. In each case, the concentration of the antagonist in the capillary was the same as that in the pond of bacteria. The viability assay for measuring survival is described in Materials and Methods.

\(^{b}\) Some of these results with α-conotoxin GVIA come from the work of Tisa, Olivera, and Adler (59). The concentrations stated in the parentheses represent the results obtained with Tris-EDTA-treated cells. This treatment allows the free passage of ions to the cytoplasmic membrane.

\(^{c}\) Gallopamil, also called D-600, is methoxyverapamil. Tiapamil is another verapamil derivative; it is a calcium channel blocker with cardiovascular effects (18).

\(^{d}\) In animals, a Na\(^{+}\) channel blocker (8, 23).

\(^{e}\) In animals, a K\(^{+}\) channel blocker (8, 23).
measured; in addition, the dose required to reduce survival by 50% was determined (Table 1).

**Calcium blockers.** A variety of Ca$^{2+}$ channel blockers in animals was tested (Table 1). ω-Conotoxin GVIA (here called ω-conotoxin) is a peptide in the venom of cone shells which in animals specifically blocks Ca$^{2+}$ channels at picomolar concentrations (35). Figure 1 shows that ω-conotoxin was highly effective in inhibiting chemotaxis as previously reported (59); chemotaxis was up to 100 times more sensitive to ω-conotoxin than was motility (Fig. 1; Table 1, see column 3). Gallopamil (called also D-600), verapamil, diltiazem, tiapamil, nifedipine, and nitrendipine block Ca$^{2+}$ channels of animals at 20 nM to 50 μM (23), but they are not perfectly selective for Ca$^{2+}$ channels, and so high concentrations can depress Na$^{+}$ and K$^{+}$ channel currents as well (23); all those compounds are modeled after papavarine, a muscle relaxant found in opium (23). Gallopamil inhibited chemotaxis 100 times more effectively than it inhibited motility, though higher concentrations were needed for gallopamil than for ω-conotoxin. Verapamil was a close third, and diltiazem and tiapamil ranked next. None of these listed above harmed viability at the highest concentrations tested. Then ranked chlorpromazine, tetracaine, and nifedipine, each inhibiting chemotaxis 10 times more effectively than motility. In the remainder of the list (Table 1, see column 3) are Ca$^{2+}$ antagonists which inhibited chemotaxis not much better than they inhibited motility.

The effect of these drugs on swimming behavior was tested. Normally, *E. coli* alternately runs and tumbles. Cells treated with each of these drugs swam with a running bias, i.e., running was predominant and tumbling was inhibited. To provide an objective analysis of these effects, the observations were subjected to computer analysis. Microscopic observations of free-swimming bacteria were videotaped, digitized, and analyzed (48). This technique measures both the average angular speed (the rate of change in direction) and the average linear speed (the rate of movement in a straight line) of a population of motile bacteria. The angular and linear speeds are directly and inversely proportional, respectively, to the tumbling frequency: an increase in the angular speed reflects an increase in the time spent tumbling, while a decrease corresponds to reduction in tumbling. The results are presented in Fig. 2. All of the Ca$^{2+}$ blockers tested as reported in Table 1 inhibited tumbling and thus promoted a running bias which lasted as long as the blocker was present (unlike in the case of attractants, where pure running stops due to adaptation). There was no reduction in running speed, and so there should be no problem with reduced proton motive force.

The inhibition of chemotaxis by verapamil (Table 1) can be completely overcome by addition of Ca$^{2+}$ (Fig. 3). Mg$^{2+}$, even more effectively than Ca$^{2+}$, overcame the inhibition by verapamil (Fig. 3): inhibition by 100 μM verapamil was completely overcome by 30 μM MgCl$_2$ compared to 300 μM CaCl$_2$. (The...
chloride are known sometimes to act differently from each other (see Table 2 on p. 131 of reference 23). In E. coli, 4-aminopyridine completely eliminated chemotaxis at 10 mM and had no significant effect at 1 mM or less, while the motility assay was not influenced at 10 mM (Fig. 5). Tetraethylammonium chloride had no effect on either chemotaxis or motility at 10 mM.

**DISCUSSION**

This study has shown that organic antagonists for the action of calcium ions, sodium ions, or potassium ions in animals have inhibitory effects on E. coli chemotaxis. The most effective Ca\(^{2+}\) antagonist that we have found for bacterial chemotaxis is \(\omega\)-conotoxin GVIA, the most effective Na\(^{+}\) antagonist is saxitoxin, and the K\(^{+}\) antagonist is 4-aminopyridine. See Results for a discussion of how these antagonists act in animals.

While animal-like K\(^{+}\) channels do occur in bacteria (17, 25, 29, 38, 49, 67), animal-like Ca\(^{2+}\) channels and animal-like Na\(^{+}\) channels do not appear to be coded for by the E. coli genome (3) (see the introduction). Therefore, it is surprising that the Ca\(^{2+}\) antagonists and the Na\(^{+}\) antagonists studied here should inhibit E. coli chemotaxis. The mechanism of inhibition by the various antagonists needs to be determined.

It is clear that the Ca\(^{2+}\) antagonists all block tumbling and promote running, while the Na\(^{+}\) antagonist saxitoxin does the opposite. How this is accomplished is not known. One possibility is that the methyl-accepting chemotaxis proteins are themselves ion channels, or else the methyl-accepting chemotaxis proteins could signal to a separate ion channel (or channels). A second possibility is that the complex of methyl-accepting chemotaxis proteins-CheW-CheA-CheY or -CheY-phosphate could signal to an ion channel (or channels). A third possibility is that

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**FIG. 3.** Ca\(^{2+}\) or Mg\(^{2+}\) overcomes inhibition of chemotaxis by verapamil (strain AW574). In the capillary assays, l-aspartate (10\(^{-2}\) M) was present as an attractant in the capillary and verapamil (10\(^{-4}\) M), together with various concentrations of CaCl\(_2\) (closed squares) or MgCl\(_2\) (open squares), was present in both the capillary and the cell suspension. Incubation was at 30°C for 1 h. The uninhibited response (10 mM K\(^{+}\) phosphate [pH 7.0] and 0.1 mM K\(^{+}\) EDTA, no verapamil, no CaCl\(_2\), and no MgCl\(_2\)) was 47,800 (±8%) bacteria in the experiment for CaCl\(_2\) and 230,000 (±8%) bacteria in the experiment for MgCl\(_2\); the difference between the two is due to variation in results that was obtained between the two different days when experiments were performed.

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**FIG. 4.** Effect of saxitoxin on bacterial chemotaxis by chemotactically wild-type E. coli (strain RP487). The procedure was as described for Fig. 1, except that there was no preincubation. The capillaries contained 10\(^{-2}\) M K\(^{+}\) l-aspartate. (To avoid K\(^{+}\), we tried l-serine instead of K\(^{+}\) l-aspartate as the attractant; similar results were obtained.) The pond of bacteria contained the same chemotaxis medium as did the capillary. In each case, the concentration of saxitoxin in the pond of bacteria was the same as that in the capillary.
the antagonists do not block ion channels at all in bacteria but rather act in some different ways.

The ion channel hypothesized above could allow all three ions, Ca$^{2+}$, Na$^+$, and K$^+$, to move through it, much as is done in animals by the acetylcholine receptor channel, the cGMP-gated visual receptor channel, or the cyclic AMP-gatedolfaction receptor channel (23). Alternatively, there could be separate channels for each of these ions: a Na$^+$ channel and a K$^+$ channel perhaps to allow a change in membrane potential, similar to the typical eukaryotic action potential, and then a Ca$^{2+}$ flux to signal the flagella to bring about tumbling, much as there is signalling by Ca$^{2+}$ at the end of a nerve cell. There is evidence that CheY-phosphate interacts with the Fli proteins at the base of the flagellum to bring about clockwise rotation and thus tumbling (6, 46, 63); a change in membrane potential could be used to introduce Ca$^{2+}$ at that site.

It is interesting in this regard that Metzner wrote in 1920 (translated), “The transmission [in Spirillum volutans] is usually so fast that both flagellar bundles [one bundle at each end of the cell] are reversed practically simultaneously” (p. 409 in reference 37). Krieg et al., working with flagellar coordination in S. volutans, have stated: “The transmission of the cell... is evidence that CheY-phosphate interacts with the Fli proteins at the end of the cell.” We have shown that simultaneous reorientation of flagellar fascicles occurs in normal cells of S. volutans. Certainly a variety of information will be required to illuminate further the fascinating frontier of bacterial ‘nervous systems.’” (27). However, Caraway and Krieg have strongly suggested that action potentials are not a factor in the flagellar coordination mechanism of S. volutans but that the cell membrane may still be the site of the mechanism (9).

For E. coli, the idea of an action potential for the flagella has not received support; there is evidence for only a short-range signalling system for the flagella (50). Our recent work on filaments (“snakes”) of E. coli is in agreement with this conclusion: an action potential seems unnecessary because all along the filament there are clusters of chemoreceptors able to communicate with the nearby flagella by means of CheY-phosphate (30).

Since S. volutans (9, 27, 37) and S. aurantia (20) are 20 to 50 μm long, they may require an action potential while smaller bacteria do not, but for smaller bacteria (about 2 μm long) such as E. coli (16) and B. subtilis (33) calcium channels have been found, and for E. coli (25, 38), S. lividans (17, 29, 49), and the cyanobacterium Synechocystis spp. (13), K$^+$ channels have been reported and characterized. Ion channels may be important for motility and chemotaxis, even when an action potential does not occur.

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