Volume 236, number 2, 437-440 FEB 06259 August 1988

Calcium channel blockers inhibit bacterial chemotaxis

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Received 8 July 1988

The effect of several Ca^{2+} channel blockers, which inhibit the voltage-dependent Ca^{2+} uptake in *Bacillus subtilis*, on chemotactic behaviour of the bacterium was studied. Nitrendipine, verapamil, $LaCl_3$ and ω-conotoxin were tested and these blockers inhibited chemotactic behaviour in the bacterium toward L-alanine. Among these blockers, ω-conotoxin was the most effective inhibitor of chemotaxis. EGTA was also as effective as ω-conotoxin. In contrast, these blockers, did not inhibit the motility and the growth of the bacterium. These results suggest that internal Ca^{2+} plays an important role in the sensory system of bacterial chemotaxis.

Ca^{2+} channel blocker; ω-Conotoxin; Chemotaxis; Ca^{2+}; (*Bacillus subtilis*)

1. INTRODUCTION

In eukaryotes, Ca^{2+} plays an important role in the regulation of a number of motile processes such as muscular contraction [1], cytoplasmic contraction [2] and ciliary reversal [3]. Ca^{2+} enters the cell through a voltage-dependent Ca^{2+} channel, which is specifically antagonized by Ca^{2+} channel blockers. In bacteria, the Ca^{2+} influx is also voltage dependent [4], and as described in the previous paper, the voltage-dependent Ca^{2+} uptake system in *Bacillus subtilis* is sensitive to Ca^{2+} channel blockers [5]. However, the role of internal Ca^{2+} in bacterial cells is not fully understood. Recently, it was reported that Ca^{2+} regulates chemotactic behaviour in *B. subtilis* [6], but the phenomenon has not been fully convincing. Here, we examined the effect of Ca^{2+} channel blockers of chemotaxis and motility in *B. subtilis* to investigate the role of Ca^{2+} in chemotactic behaviour.

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2. MATERIALS AND METHODS

2.1. *Motile bacteria*

Motile cells of *Bacillus subtilis* W23, *Ade*$^-$, *Met*$^-$ were prepared as described by Ordal and Goldman [7]. *B. subtilis* was grown in Spizizen's minimal medium [8] containing 25 μM adenine and 250 μM methionine at 37°C. At the stationary phase, the cells were diluted 1:50 into mineral salt medium [7] containing 20 mM sorbitol, 0.3 mM L-alanine and required nutrients, grown at 37°C with shaking to an A_{660} value of 0.3 (early log phase), and then made 5 mM in sodium lactate and 0.05% in glycerol. After 15 min, the bacteria were centrifuged and washed twice with chemotaxis buffer (0.01 M potassium phosphate, pH 7.0, 0.14 mM $CaCl_2$, 0.3 mM $(NH_4)_2SO_4$, 0.1 mM EDTA, 5 mM sodium lactate and 0.05% glycerol).

2.2. *Chemotaxis assay*

2.2.1. Swarm agar plate method [9]

About 2×10^7 motile cells were deposited at the center of a swarm agar plate (0.2% agar) of mineral salts medium containing 2.0 mM L-alanine and a channel blocker. Photographs were taken after 10 h of incubation at 37°C.

2.2.2. Capillary method

Bacteria were washed and diluted with the chemotaxis buffer to a cell concentration of 3.5×10^6 cells per ml (bacterial suspension). Capillary assays were performed by the method of Adler [10] using 2-μl microcapillaries at 37°C.

2.3. *Measurement of motility*

Motility of the bacterium was observed as described by Shioi

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

et al. [11]. The cells were suspended in the chemotaxis buffer to a concentration of 2×10^6 cells per ml. The movement of the bacteria on a glass slide was observed at 30°C with a dark field microscope and recorded with a high-speed videotape MHS-200 (nac, Japan). Swimming speed of the cells was measured from photographs taken from the videoscenes with an exposure time of 1 s.

3. RESULTS AND DISCUSSION

Previously, we have shown that the voltage-dependent uptake of Ca^{2+} in the membrane vesicles of *B. subtilis* was inhibited by Ca^{2+} channel blockers such as nitrendipine (10 μM) or verapamil (25 μM) [5]. In addition to these two kinds of blockers, a novel specific Ca^{2+} channel blocking peptide, ω-conotoxin, which has been recently isolated [12] and synthesized by the Peptide Institute, Protein Research Foundation (Japan) [13,14], was also used for the present study. ω-Conotoxin (5 μM) also inhibited 60% of the Ca^{2+}-uptake activity in the membrane vesicles of this organism (not shown). However, these blockers had no effect on the growth of *B. subtilis* even when the concentrations of these drugs (e.g., 50 μM of nitrendipine or 5 μM of ω-conotoxin) were higher than those sufficient for the complete inhibition of Ca^{2+} uptake (not shown).

We tested the effect of these drugs on chemotaxis in a semi-solid agar plate containing 2 mM L-alanine as an attractant. After 10 h of incubation at 37°C, the diameters of the rings which were formed by the migrated bacteria were directly compared. As shown in fig.1, the migration was inhibited 70% by 5 μM ω-conotoxin, 52.5% by 0.5 μM ω-conotoxin, 40% by 100 μM verapamil, 52.5% by 25 μM nitrendipine and 32.5% by 10 μM nitrendipine. Inhibitory concentrations of these blockers were comparable to those for Ca^{2+}-uptake activity as described above.

The chemotaxis of *B. subtilis* was also assayed

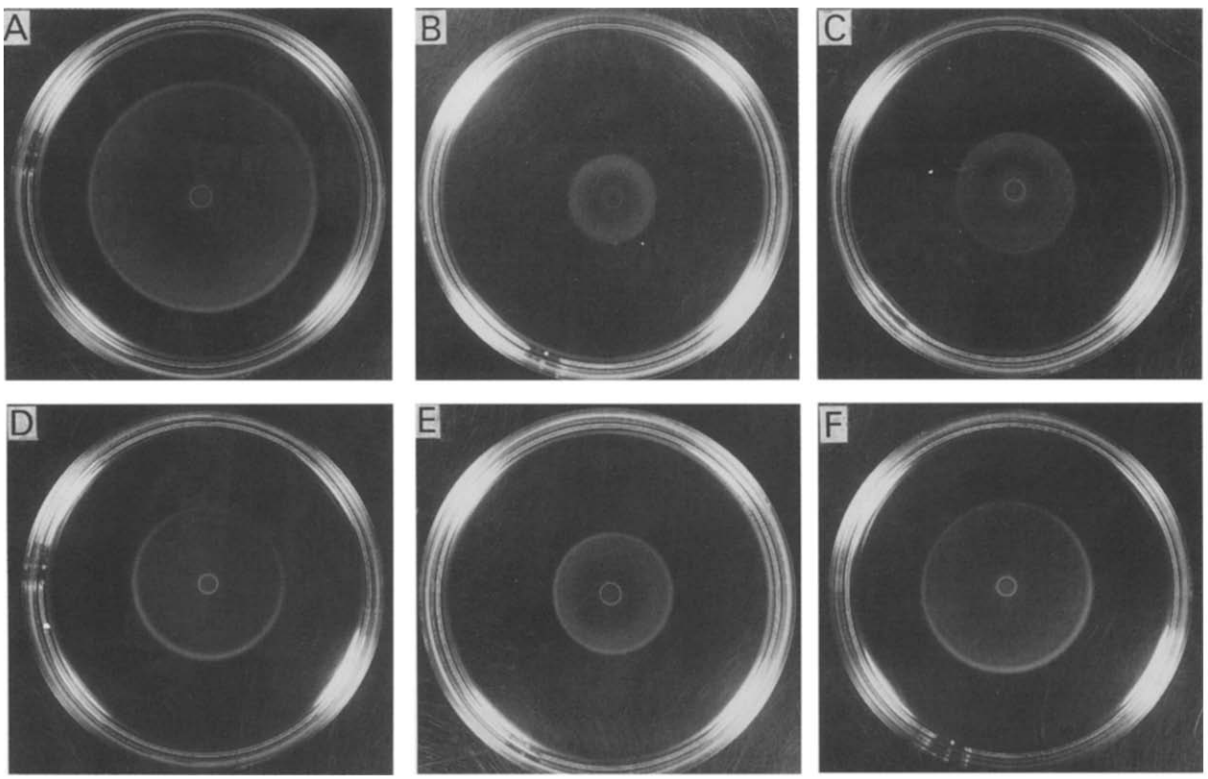

Fig.1. Effect of Ca^{2+} channel blockers on chemotaxis in *Bacillus subtilis* toward L-alanine assayed by the swarm agar plate method. Each agar plate contained 2.0 mM L-alanine (A, control) and in addition, 5 μM ω-conotoxin (B); 0.5 μM ω-conotoxin (C); 100 μM verapamil (D); 25 μM nitrendipine (E); or 10 μM nitrendipine (F) was supplemented.

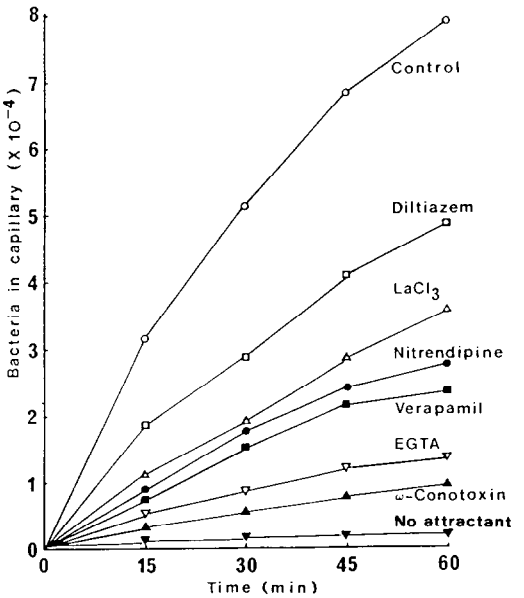

Fig.2. Effect of Ca^{2+} channel blockers on chemotaxis in *Bacillus subtilis* assayed by the capillary method. A capillary containing 5 mM L-alanine is inserted into a bacterial suspension in chemotaxis buffer (○), control. A capillary containing no attractant (▼). When the effect of channel blockers on the chemotaxis was assayed, each drug was added both in the capillary and in the bacterial suspension. (□) 100 μM diltiazem; (△) 100 μM $LaCl_3$; (●) 10 μM nitrendipine; (■) 100 μM verapamil; (▽) 10 mM EGTA; (▲) 1 μM ω-conotoxin.

by the capillary method. Fig.2 shows the effect of channel blockers on the migration of cells into the capillary containing 5 mM L-alanine as an attactant. When the capillary contained no attractant (the bottom curve, none), a very small number of bacteria entered the capillary (randomly swimming cells). When the capillary contained 5 mM L-alanine, motile bacteria were attracted into the capillary without any lag, and 8×10^4 cells were found in the capillary after 60 min. In the presence of 1 μM ω-conotoxin, both in the capillary and in the bacterial suspension, only 4.5×10^3 cells had migrated into the capillary after 60 min, which corresponded to more than 90% inhibition of the chemotaxis. Almost the same effect was found with 10 μM nitrendipine (57% inhibition) and 100 μM verapamil (64% inhibition). Diltiazem, another Ca^{2+} channel blocker, also inhibited the migration (about 40% by 100 μM of the drug). On the other hand, 10 mM EGTA was as effective as 1 μM ω-conotoxin. Since the media contained 0.14 mM of Ca^{2+}, the free Ca^{2+} concentration was

Table 1

Effect of Ca^{2+} channel blockers on the motility of *Bacillus subtilis*

Ca^{2+} channel blocker	Concentration (M)	Swimming speed[a] (μm/s)
None	–	29.4 ± 5.8
ω-Conotoxin	1×10^{-5}	28.6 ± 4.1
Nitrendipine	5×10^{-5}	30.4 ± 5.5
$LaCl_3$	2.5×10^{-4}	30.0 ± 3.6
EGTA	1×10^{-2}	31.5 ± 7.5

[a]Values are the mean ± one standard deviation

Measure of motility was carried out with more than 100 tracks as described in section 2. A correction of the cell size (about 7 μm in average) was made to obtain the swimming speed of the cell

calculated to be 2.2 nM (based on the equation described in [6]) in the presence of 10 mM EGTA; therefore, the bacterium could not respond to the attractant when the free Ca^{2+} concentration was too low.

The effect of Ca^{2+} channel blockers on the motility of the bacterium was observed under a television monitor and is shown in table 1. Cells of *B. subtilis* moved about 30 μM/s (control), and the values were almost the same in the cells containing 5 μM ω-conotoxin, 50 μM nitrendipine, 250 μM $LaCl_3$ and 10 mM EGTA. Therefore, it was concluded that these blockers did not inhibit the motility of the bacterium.

Thus, various Ca^{2+} channel blockers inhibit chemotactic behaviour without any effect on either cell growth or motility. These results suggest that internal Ca^{2+} plays an important role in the sensory system of bacterial chemotactic behaviour, and they also suggest that Ca^{2+} may flow into bacterial cells through a Ca^{2+} channel-like system as in the excitable membranes of eukaryotes.

Acknowledgements: We thank the Peptide Institute, Protein Research Foundation (Japan) for providing the synthetic ω-conotoxin. The synthetic ω-conotoxin was developed through a collaboration between the Peptide Institute, Protein Research Foundation and the organizing group of Special Project Research on the Mechanism of Bioelectrical Response, which is supported by a Grant-in-Aid (61107004) from the Japanese Ministry of Education, Science and Culture.

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