Inhibitory Effect of Beauvericin on a High K⁺-Induced Tonic Contraction in Guinea-Pig Taenia Coli

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Abstract—An inhibitory effect of beauvericin, a cyclodepsipeptide, on a high K⁺-induced contraction in guinea-pig taenia coli was compared with those of verapamil, an organic Ca²⁺ antagonist, and monensin, an inhibitor of mitochondrial respiration. Beauvericin (10⁻⁵ M), verapamil (5×10⁻⁷ M) or monensin (10⁻⁶ M) markedly inhibited the tonic contraction, while these drugs showed less effect on the phasic contraction. Beauvericin at a lower concentration (10⁻⁶ M) competitively inhibited the Ca²⁺-induced contraction in depolarized muscle, whereas higher concentrations (3×10⁻⁶ or 10⁻⁵ M) non-competitively inhibited this contraction. Verapamil (10⁻⁸–5×10⁻⁷ M) competitively inhibited and monensin at a low concentration (10⁻⁷ M) non-competitively inhibited this contraction. A contraction induced by 0.5 mM Ca²⁺ was inhibited by beauvericin with an IC₅₀ (concentration needed for 50% inhibition) of 2.8×10⁻⁷ M, verapamil with an IC₅₀ of 2.9×10⁻⁸ M, and nifedipine with an IC₅₀ of 1.8×10⁻⁹ M. 10⁻⁶ M CGP 28392, a Ca²⁺ channel facilitator, increased the IC₅₀ of beauvericin, verapamil or nifedipine. Although the inhibitory effect of monensin (10⁻⁷–10⁻⁶ M) on the high K⁺-induced contraction was reduced under hypoxia, the effects of beauvericin (10⁻⁷–10⁻⁵ M) and verapamil (10⁻⁸–10⁻⁷ M) were not modified. Beauvericin (10⁻⁶ M) changed neither the intracellular Na⁺ and K⁺ contents of the depolarized muscle nor the Ca²⁺-induced contraction in the chemically skinned taenia coli. These results suggest that the inhibitory action of beauvericin (10⁻⁶ M) on the high K⁺-induced tonic contraction is due to the non-competitive inhibition of Ca²⁺ entry through the voltage-dependent Ca²⁺ channel of the intestinal smooth muscle cell.

It is known that a number of fungal species cause diseases in insects, and some of them produce toxic substances. For example, Aspergillus flavus which attacked silkworm larvae produced aflatoxin (1). Murakoshi et al. (2) isolated two fungal species, Beauveria bassiana and Verticillium lecanii, from the diseased pupae of the silkworm, Bombyx mori L, and they obtained two toxic substances from mycelia of these fungi, which were designated as bassianolide and beauvericin. Nakajyo et al. (3, 4) have reported that bassianolide inhibits contractions induced by receptor agonists (i.e., acetylcholine or histamine), but not contractions induced by agents which affect non-selective sites of the muscle cell (i.e., high K⁺ or barium). Although beauvericin is known to be a selective ionophore to monovalent cations (5–7), an effect of beauvericin on smooth muscles has not been reported.

In the present study, we investigated the effect of beauvericin on the high K⁺-induced contraction of guinea-pig taenia coli, and we compared the effect of beauvericin with those of verapamil, an organic Ca²⁺ antagonist, and monensin, which is an ionophore to monovalent cations and shows an inhibitory effect on mitochondrial respiration in...
Male guinea-pigs weighing 300–400 g were bled after stunning. Smooth muscle preparations of taenia coli were dissected from the caecum and immediately soaked in a normal physiological solution (PSS). The composition of the PSS was as follows (mM): NaCl, 136.8; KCl, 5.4; CaCl₂, 2.5; MgCl₂·6H₂O, 1.0; NaHCO₃, 11.9 and glucose, 5.5. The bathing solution was bubbled with a gas mixture of 95% O₂ and 5% CO₂, and it had a pH of 7.2 at 37°C. A hypoxic condition was produced by bubbling the bathing medium with a gas mixture of 95% N₂ and 5% CO₂.

The muscle strip was suspended in an organ bath containing 15 ml of PSS and one end of the muscle was connected with a holder and another to the arm of a strain-gauge transducer (Nihon Kohden, SB-1TA) with silk thread. The output was displayed on a linear recorder (Nihon Kohden, RJG-4008), and muscle tension was recorded isometrically. The tension loaded to the strip was about 0.5 g. Sixty mM KCl was hyperosmotically applied twice for 15 min to the muscle after 60 min incubation in PSS. The maximal tension of the phasic or tonic contraction induced by the second application of the high K+ (65.4 mM) solution was regarded as a reference response (100%). The effect of drug was also investigated on a contraction induced by adding Ca²⁺ to the muscles in Ca²⁺-free, high K⁺ solution (a Ca²⁺-induced contraction). The Ca²⁺-free solution was made by omitting CaCl₂ from the original medium without any substitution for osmolarity. An elevation of Ca²⁺ concentration above 10 mM was made by substituting a Tris-HCl buffer system for the NaHCO₃-CO₂ one in the PSS, and this solution was bubbled with 100% O₂ gas.

Intracellular Na⁺ and K⁺ contents were determined as reported by van Breemen et al. After incubation with a test solution, the muscle was exposed to a La³⁺-solution (294.9 mM sucrose, 1.0 mM LaCl₃ and 11.9 mM Tris-HCl) at 0.5°C. After a 30 min-washout of the tissues, they were gently blotted between filter papers, weighed, and ashed in a quartz tube with 0.5 ml of a mixture (1:1) of HNO₃ (61%) and HClO₄ (60%), overnight at 180°C. The dried samples were then dissolved in 0.01 N HCl solution. CsCl (1 g/l) was added to standard and diluted solutions to inhibit a mutual interference of Na⁺ and K⁺. The ion concentration of the diluted sample was measured with a flame photometer (Varian, Type AA-275).

In another series of experiments, the effect of the drug was tested on a saponized muscle. A small bundle of the muscle fibers, 100–150 μm in width and several milimeters in length, was cut with Weckels scissors under a microscope in the PSS. One end of the fiber was connected to a holder silk thread and the other end to a strain-gauge transducer (Nihon Kohden, SB-1TA); then isometric tension was recorded. After a high K⁺-induced contraction was recorded, the solution was changed to a “standard relaxing solution” containing (mM) KCl, 130; Tris-maleate, 20; MgCl₂·6H₂O, 5; ATP-Na₂, 5 and EGTA, 2 (pH 6.8). Saponin treatment was then carried out by keeping the specimen for 20 min in standard relaxing solution containing 100 μg/ml saponin. These procedures were almost the same as those described by Saida and Nonomura.

Beauvericin isolated from cultured fungi, Beauveria bassiana, is a cyclodepsipeptide composed of L-N-methyl phenylalanine and D-2-hydroxyisovaleric acid. Its molecular formula is C₄₅H₅₇N₃O₉ and its molecular weight is 783. Beauvericin is a white crystalline substance which is insoluble in water but soluble in organic solvents. Beauvericin dissolved in ethanol solution was applied in a volume of less than 0.02 ml to the 15 ml organ bath solution. The same volume of ethanol was applied as a control to determine whether ethanol itself has any influence upon the preparation in any of the experiments on this study. The sample was kindly donated by Dr. Suzuki, Department of Agricultural Chemistry, Faculty of Agriculture, the University of Tokyo.

The drugs used were verapamil (Eisai), CGP 28392 (Ciba-Geigy), nifedipine (Sigma) and saponin (ICN).

The results were expressed as mean values ± S.E. Statistical significance was determined...
Results

Effect of beauvericin on high K⁺-induced contraction: When various concentrations (10⁻⁷–10⁻⁵ M) of beauvericin were applied, they did not change spontaneous contraction of the smooth muscle in guinea-pig taenia coli. An elevation of potassium concentration to 65.4 mM in the PSS induced a phasic contraction that was followed by a tonic one. The muscle tension in the phasic response was approximately 7–10 g and that in the tonic one, 8–11 g (Fig. 1). 10⁻⁷–10⁻⁶ M beauvericin slightly inhibited the phasic component of the contraction induced by repeated application of high K⁺ for 15 min at 15 min interval, and 10⁻⁵ M beauvericin inhibited it to about a half within 15 min (Figs. 1 and 2A). Beauvericin at 10⁻⁷ M did not affect the tonic component of high K⁺-induced contraction, but 10⁻⁶ M beauvericin gradually decreased the muscle tensions to 60% at 90 min. Beauvericin at 10⁻⁵ M completely inhibited the tonic contractions at 90 min (Figs. 1 and 2B). Verapamil at 5×10⁻⁷ M inhibited the phasic component of the high K⁺ contraction to 60% within 45 min and almost completely inhibited the tonic one at 30 min. Monensin at 10⁻⁶ M also almost completely inhibited the tonic contraction at 30 min but did not affect the phasic ones (Figs. 1, 2C and D).

In the next series of experiments, 10⁻⁷–10⁻⁵ M beauvericin was added to the medium at 30 min after an application of high K⁺.

Fig. 1. Effect of beauvericin (10⁻⁵ M) verapamil (5×10⁻⁷ M) or monensin (10⁻⁶ M) on contractions induced by repeated applications of hyperosmotic 65.4 mM K⁺ in guinea-pig taenia coli. High K⁺ was applied for 15 min and the wash period was 15 min.
Beauvericin at $10^{-5}$ M caused a gradual decline of the K$^+$-induced contraction, and muscle tension reached a new steady level of approximately 0.2 g within 120 min. Beauvericin at $10^{-7}$ and $10^{-6}$ M also decreased the developed tension to 87% and 48% of the control value, respectively, in a dose-dependent manner. The after-treatment with verapamil ($10^{-8}$ and $10^{-7}$ M) and monensin ($10^{-7}$ and $10^{-6}$ M) also inhibited the high K$^+$-induced tonic contraction in more rapidly than that with beauvericin did. Data on the post-treatment with the three drugs to the high K$^+$ contraction was not shown in the figure.

Effect of beauvericin on the Ca$^{2+}$-induced contraction: Inhibitory effects of the three drugs were further investigated on the Ca$^{2+}$-induced contraction in high K$^+$-depolarized muscle. The muscle was fully relaxed after 2 hr incubation with the Ca$^{2+}$-free solution containing 65.4 mM KCl. A cumulative addition of Ca$^{2+}$ (0.25–10 mM) produced a stepwise contraction in the depolarized muscle. A pretreatment with beauvericin for 90 min, verapamil or monensin for 30 min inhibited the Ca$^{2+}$-induced contraction. The mode of inhibition by beauvericin, verapamil or monensin was estimated by Lineweaver-Burk plots (11) of the reciprocal of the rate of the Ca$^{2+}$-induced contraction against the reciprocal of the external Ca$^{2+}$ concentration.
The values in the figure gave straight lines in the presence or absence of $10^{-6}$ M beauvericin. Both of the lines showed an intersection at a single point on the ordinate. However, the straight lines by $3 \times 10^{-6}$ and $10^{-6}$ M beauvericin did not intersect with control at the same point (Fig. 3A). In the case of verapamil treatment, all straight lines intersected with the control line at the same point on the ordinate, indicating that the antagonism of verapamil to external $Ca^{2+}$ is typically competitive (Fig. 3B). The line for $10^{-6}$ M monensin almost rectangularly intersected on the ordinate, and the result seems to show that the inhibition is not related to external $Ca^{2+}$. However, the lines for $10^{-7}$ M monensin and the control intersected at different points on the ordinate, indicating that its inhibition is non-competitive (Fig. 3C). Thus, the inhibition of $10^{-6}$M beauvericin on the $Ca^{2+}$-induced contraction seems to be a competitive antagonism to external $Ca^{2+}$, but that of beauvericin at the higher concentration of $3 \times 10^{-6}$ or $10^{-5}$ M was non-competitive.

The effect of CGP 28392, a $Ca^{2+}$ channel

![Figure 3](image-url)

**Fig. 3.** Lineweaver-Burk plot of data obtained from inhibition of $Ca^{2+}$-induced contraction by beauvericin (A), verapamil (B) or monensin (C). Ordinate: reciprocal of rate of $Ca^{2+}$-induced contraction, $1/(tension/min)^{-1}$. Abscissa: reciprocal of external $Ca^{2+}$ concentration, $1/(Ca^{2+} \text{ mM})^{-1}$. Data were obtained at 90 min after beauvericin application and at 30 min after verapamil or monensin application. Each point represents the values from 5–6 experiments.
facilitator (12), on the inhibition of beauvericin, verapamil or nifedipine to a submaximal Ca\textsuperscript{2+}-induced contraction was assessed in the depolarized muscle. An application of 0.5 mM Ca\textsuperscript{2+} to a Ca\textsuperscript{2+}-free high K\textsuperscript{+} solution caused a submaximal contraction, to 76% of that induced by 60 K\textsuperscript{+} (100%). Ca\textsuperscript{2+} at 0.5 mM was added at 90 min after a beauvericin application or 30 min after a verapamil or nifedipine one. CGP 28392 (10\textsuperscript{-6} M) was applied to the muscle treated with the inhibitor at 15 min before the Ca\textsuperscript{2+} addition. CGP 28392 augmented the submaximal Ca\textsuperscript{2+}-induced contraction to 96% in the absence of these drugs. Although beauvericin, verapamil and nifedipine at various concentrations inhibited the Ca\textsuperscript{2+}-induced contraction, CGP 28392 (10\textsuperscript{-6} M) partly recovered it. The concentrations of the three drugs needed to inhibit the Ca\textsuperscript{2+}-induced contraction by 50% (IC50) are shown in Table 1. That is, the Ca\textsuperscript{2+}-induced contraction was inhibited by beauvericin with an IC50 of 2.8×10\textsuperscript{-7} M, verapamil with an IC50 of 2.9×10\textsuperscript{-8} M, and nifedipine with an IC50 of 1.8×10\textsuperscript{-9} M. CGP 28392 (10\textsuperscript{-6} M) significantly increased the IC50 of beauvericin or verapamil about 4-fold and increased the IC50 of nifedipine by about one order.

**Effect of beauvericin on the high K\textsuperscript{+} induced contraction under hypoxic conditions:** A hypoxic condition decreased the spontaneous activity of the muscle within 30 min. On the other hand, the muscle treated with high-K\textsuperscript{+} solution produced a rapid phasic contraction following by a very small tonic contraction under the hypoxic condition. The relative tension of the tonic contraction was 22±1.7% (n=12) of that under the aerobic condition. The muscle tension in the tonic response was gradually increased by addition of 40 mM glucose to the medium and reached a new steady level to 45.6±5.3% (n=8) of that of the control within 20 min. Such a contraction maintained under hypoxia was more resistant to the inhibitory effect of monensin (10\textsuperscript{-7} and 10\textsuperscript{-6} M) than that under normoxia; however, the contraction under hypoxia was inhibited by beauvericin (10\textsuperscript{-7}–10\textsuperscript{-6} M) or verapamil (10\textsuperscript{-8} and 10\textsuperscript{-7} M) to the same degree as that under normoxia. The concentration-inhibition curves in terms of the inhibitory effects of monensin and beauvericin under normoxia and hypoxia are shown in Fig. 4A, B. The concentration-inhibition curve for monensin was shifted in parallel to the right by hypoxia, while the curves for beauvericin under normoxic and hypoxic conditions were almost identical.

**Effect of beauvericin on intracellular Na\textsuperscript{+} and K\textsuperscript{+} contents:** In the smooth muscle of taenia coli, intracellular Na\textsuperscript{+} and K\textsuperscript{+} contents were 5.0±0.4 mM/kg wet wt. and 61.1±1.0 mM/kg wet wt., respectively. The high-K\textsuperscript{+} solution with or without beauvericin (10\textsuperscript{-5} M) did not significantly change the ion contents of the muscle at 15, 30 and 60 min after the treatment.

**Table 1.** IC50 of beauvericin, verapamil or nifedipine on the Ca\textsuperscript{2+}-induced contraction in the depolarized muscle of guinea-pig taenia coli, in the presence or the absence of CGP 28392 (10\textsuperscript{-6} M)

|          | Control                  | +CGP 28392 (10\textsuperscript{-6} M) |
|----------|--------------------------|----------------------------------------|
| Beauvericin | 2.8×10\textsuperscript{-7} M (n=18) | 1.3×10\textsuperscript{-6} M *(n=18) |
|           | [1.0–4.0]                | [1.2–2.1]                              |
| Verapamil | 2.9×10\textsuperscript{-8} M (n=19) | 1.4×10\textsuperscript{-7} M *(n=16) |
|           | [1.5–4.0]                | [1.3–1.6]                              |
| Nifedipine | 1.8×10\textsuperscript{-9} M (n=24) | 2.2×10\textsuperscript{-8} M *(n=23) |
|           | [1.1–2.2]                | [1.6–4.5]                              |

The Ca\textsuperscript{2+}-induced contraction was produced by an application of 0.5 mM Ca\textsuperscript{2+} to the Ca\textsuperscript{2+}-free-high K\textsuperscript{+} (65.4 mM) solution. Ca\textsuperscript{2+} at 0.5 mM was added at 90 min after beauvericin application and at 30 min after verapamil or nifedipine application. CGP 28392 was applied to the preparation treated with the inhibitor at 15 min before the Ca\textsuperscript{2+} addition. Figures in square brackets are the 95% confidence limits. *Significantly different from the control (P<0.05).
Effect of beauvericin on Ca\textsuperscript{2+}-induced contraction in chemically skinned taenia preparations: A low concentration of Ca\textsuperscript{2+} (10\textsuperscript{-7} M) contracted the chemically skinned taenia preparations which had been treated with saponin. In Fig. 5, an application of Ca\textsuperscript{2+} (10\textsuperscript{-6} M) produced a contraction to about 0.8–1.0 g, while beauvericin (10\textsuperscript{-5} M) did not significantly inhibit Ca\textsuperscript{2+}-induced contraction, and trifluoperazine (10\textsuperscript{-5} M), a calmodulin inhibitor, inhibited it to half.

Discussion

Beauvericin and bassianolide, cyclodepsipeptides, are produced by fungi, *Beauveria bassiana*, isolated from the diseased pupae of the silkworm (2). It was reported that bassianolide scarcely affected the phasic and tonic responses of the high K\textsuperscript{+}-induced contraction (4), whereas beauvericin (10\textsuperscript{-6} M) selectively inhibited the tonic response of the high K\textsuperscript{+}-induced contraction. The tonic response of high K\textsuperscript{+}-induced contraction in the polarized smooth muscle or the Ca\textsuperscript{2+}-induced contraction in the depolarized muscle is likely caused by an increase in cytoplasmic Ca\textsuperscript{2+} that entered from the external medium through a voltage-dependent Ca\textsuperscript{2+} channel (13–15). An organic Ca\textsuperscript{2+} antagonist like verapamil (16, 17) or accumulated cellular Na\textsuperscript{+} by ouabain (18, 19) and by vanadate (20) inhibited the entrance of Ca\textsuperscript{2+} through the channel. In the experiment of the Ca\textsuperscript{2+}-induced contraction, the inhibitory effect of beauvericin at the lower concentration (10\textsuperscript{-6} M) as well as that by verapamil (10\textsuperscript{-6} and 10\textsuperscript{-7} M) was competitively antagonized by raising the external Ca\textsuperscript{2+} concentration, but the effect of beauvericin at the higher concentration (3×10\textsuperscript{-6}, 10\textsuperscript{-5} M) was antagonized by Ca\textsuperscript{2+} in a non-competitive manner. Moreover, CGP 28392 (10\textsuperscript{-6} M) increased the IC50 of
beauvericin, verapamil and nifedipine on the Ca\(^{2+}\)-induced contraction. On the other hand, although beauvericin has the properties of an ionophore (5-7), it did not change the intracellular Na\(^+\) and K\(^+\) contents of the depolarized muscle. Beauvericin seems to have no influence on the permeability of monovalent cations through the smooth muscle cell membrane. Thus, it is likely that beauvericin inhibits the Ca\(^{2+}\) entry through the voltage-dependent Ca\(^{2+}\) channel without changing intracellular Na\(^+\) content.

Meanwhile, metabolic inhibiting factors such as a glucose depletion (21, 22), hypoxia (22, 23), and application of monensin (8) decreased the tonic tension of the depolarized muscle. That is, the tonic component of the high K\(^+\)-induced contraction under an aerobic condition requires energy which is produced by ATP synthesized in mitochondria of the smooth cell (23-25). In the present paper, the concentration-inhibition curve for monensin under normoxia was shifted to the right by hypoxia, as reported by Kishimoto et al. (8). However, the curves for beauvericin as well as those for verapamil under normal and hypoxic conditions were almost identical. Thus, it seems unlikely that beauvericin inhibits the high K\(^+\)-induced tonic contraction by a depression of the cell respiration, as monensin did (8). Since beauvericin damaged the teflon membrane of the Clark-type oxygen electrode, we were unable to measure the effect of beauvericin on an oxygen consumption by the smooth muscle. Beauvericin (1.5×10\(^{-5}\) M) also did not affect the Ca\(^{2+}\)-induced contraction in the chemically skinned preparation which was inhibited by trifluoperazine (10\(^{-5}\) M), a calmodulin inhibitor. Accordingly, beauvericin may not inhibit the high K\(^+\)-induced tonic contraction by a direct inhibition on contractile process(es).

In summary, beauvericin (10\(^{-5}\) M) selectively inhibited the tonic component of the high K\(^+\)-induced contraction in guinea-pig taenia coli. It is suggested that the inhibitory action of beauvericin is due to inhibition of the entry of Ca\(^{2+}\) through the voltage-dependent Ca\(^{2+}\) channel opened by depolarization mainly in a non-competitive manner, because beauvericin did not affect the intracellular Na\(^+\) content, the cell respiration and the contractile process(es).

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