INHIBITORY EFFECT OF BASSIANOLIDE, A CYCLODEPSIPEPTIDE, ON DRUG-INDUCED CONTRACTIONS OF ISOLATED SMOOTH MUSCLE PREPARATIONS

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Abstract—Bassianolide (BASS) is a cyclodepsipeptide isolated from cultured mycelia of Beauveria bassiana and is pathogenic to insects. In a longitudinal muscle preparation from guinea pig ileum, 10^-6 M BASS almost irreversibly inhibited an isotonic contraction induced by acetylcholine (ACH) and made the dose-response curve shift in parallel to the right (pA2: 7.6). It also inhibited the contractions induced by carbachol, pilocarpine, histamine, 5-hydroxytryptamine (5-HT) and prostaglandin E2, but did not inhibit the contraction induced by barium or a high concentration (40–60 mM) of potassium (high K). When applied to the guinea pig vas deferens, 10^-8–10^-7 M BASS inhibited an isometric contraction induced by norepinephrine (NE) (3×10^-8–10^-5 M), phenylephrine (3×10^-6–10^-5 M) or ACH (10^-6–10^-5 M). When the contractions of the three agonists exceeded the concentrations mentioned above, BASS failed to exert an inhibitory effect upon any of these agonists. It also inhibited the contraction caused by carbachol and histamine, but did not inhibit that induced by barium or high K. BASS itself failed to cause the contraction or relaxation of both muscle preparations. From these results, it is suggested that BASS inhibits the contraction induced by an agonist which acts upon selective sites of smooth muscle cells, but which does not inhibit a contraction induced by an agonist that has an effect on non-selective sites of cells.

A number of fungal species cause diseases in insects. Some of them are known to produce toxic substances. For example, Aspergillus flavus produces aflatoxins (1) and Beauveria bassiana produces beauvericin (2). Murakoshi et al. (3) isolated two fungal species, Beauveria bassiana and Verticillium lecanii, from the diseased pupae of the
silkworm, *Bombyx mori* L, and these are pathogenic for insects. A toxic substance was isolated from the mycelia of these fungi and it was called bassianolide (abbreviated as BASS). BASS is a white crystalline substance with a molecular formula of \((\text{C}_{12}\text{H}_{21}\text{N}_{03})_4\). It is a cyclodepsipeptide with a molecular weight of 908 (4, 5). When this substance was orally administered to the larvae of silkworms, they showed atony, or a symptom of muscular relaxation, and died eventually (3). No papers have been published as yet on the pharmacological action of BASS. The present investigation was carried out to study the effect of BASS on the contractile responses induced by various kinds of neurotransmitters and autacoids. The two smooth muscle preparations from the guinea pig used were the ileal longitudinal muscle layer and the vas deferens to obtain a cholinergic response and an adrenergic one, respectively.

**MATERIALS AND METHODS**

**Preparation of isolated ileal longitudinal muscle of guinea pig:** Male guinea pigs weighing 400–500 g were killed by a blow on the head and bled to death. A length of small intestine was removed, about 10 cm of the terminal ileum being discarded. The longitudinal muscle was prepared by the method described by Paton and Abbo Zar (6). The muscle preparation, about 1 cm long, was suspended in a modified Tyrode solution exposed to a mixture of 95% oxygen and 5% carbon dioxide. The composition of this solution (mM) was NaCl, 136.8; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.0; NaH₂PO₄, 0.4; NaHCO₃, 11.9; and glucose, 5.5. The organ bath was kept at 36±1 °C and at pH 7.2. The contraction of the ileal longitudinal muscle was recorded isotonically on a piece of smoked paper on a kymograph. A resting tension of 0.5 g was loaded. The magnification was ten times. The muscle preparation was suspended in an organ bath for about 1 hr until the tonus of the muscle was stabilized. Then 1 ml of 1M KCl solution was added hypotonically to the 25 ml organ bath to give a final concentration of 40 mM K. The maximal size of the muscle contraction shown in 10 min was regarded as 100%. The size of muscle contraction in response to each agonist was expressed as the contraction (%) relative to the maximal contraction of the muscle exposed to 40 mM K solution. Drugs were cumulatively added to the medium in the organ bath to obtain a dose-response curve. Antagonists or BASS were applied 15 min before the application of agonists.

**Preparation of isolated vas deferens:** The vas deferens was excised, and the serous membrane was carefully stripped away. The middle part of the strip, about 2 cm long, was suspended in a 15 ml organ bath containing Krebs solution of the following composition (mM): NaCl, 118.0; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgCl₂, 1.2; NaHCO₃, 25.0; and glucose, 1.1. The organ bath was aerated with 95% oxygen and 5% carbon dioxide and kept at 32±1 °C. A resting tension of 1 g was applied to each strip. The preparations were equilibrated for 60 min before application of drugs. The tension was measured isometrically by a force-displacement transducer and recorded on a polygraph (Nihon Kohden Co. Ltd). When the muscle of the preparation became steady, each drug was applied for 5 min. After a drug was washed out, the drug was repeatedly applied at 30 min intervals. The antagonists used were pretreated for mostly 15 min. The preparation of the vas deferens was applied with a single dose of each drug in various concentrations and not with cumulative doses, since the response of the tissue to the drug was phasic. Then a dose-response curve was made.

**Bassianolide:** BASS is a white crystalline
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Fig. 1. Structure of bassianolide.

substance isolated from the cultured fungi Beauveria bassiana. It is a cyclodepsipeptide composed of four molecules of 1-N-methyl-leucine and d-a-hydroxy-isovaleric acid. Its molecular formula is \((C_{12}H_{21}NO_3)_4\) and its molecular weight is 908 (Fig. 1). It is insoluble in water and soluble in organic solvents (4). BASS was dissolved in ethanol at the desired concentration and the ethanol solution was applied in a volume of less than 0.02 ml in the 15 ml organ bath solution. The same volume of ethanol was applied as a control to confirm that ethanol itself had no influence upon the preparation.

Drugs: The following drugs were used for the experiment: acetylcholine chloride (ACH; Daiichi Pharmaceutical Co.), 1-acetyl-2-(8-chloro-10,11-dihydrodibenz \([b,f\]) \([1,4\] oxazepine-10-carbonyl)hydrazine (SC-19220; Searle & Co.), atropine sulfate (ATR; Wako Pure Chemicals Co.), BaCl\(_2\) (Ba; Wako Pure Chemicals Co.), carbachol chloride (Sigma), histamine dihydrochloride (Wako Pure Chemicals Co.), morphine hydrochloride (Daiichi Pharmaceutical Co.), norepinephrine bitartrate (NE; Sigma), papaverine hydrochloride (Wako Pure Chemicals Co.), phenylephrine hydrochloride (Tokyo Kasei Co.), pilocarpine hydrochloride (Tokyo Kasei Co.), prostaglandin E\(_2\) (PGE\(_2\); Ono Pharmaceutical Co.), 5-hydroxytryptamine (5-HT; Wako Pure Chemicals Co.), tripelennamine (CIBA) and phentolamine (Regitin, CIBA).

The potency of each drug was expressed as \(pA_2\) and \(pD_2\) (7, 8). The results were analyzed using the Student’s \(t\)-test to determine significant difference.

RESULTS

The ileal longitudinal muscle preparation: When \(10^{-8}\)–\(10^{-6}\) M BASS was applied, the ileal longitudinal muscle of guinea pig did not contract or relax. ACH (3\(\times\)10\(^{-8}\) M) immediately induced a contraction which was inhibited by 1\(\times\)10\(^{-7}\) M ATR. Pretreatment with 10\(^{-6}\) M BASS inhibited the ACH induced contraction similarly to an extent of 20% (Fig. 2-A, A’). A dose-response curve was prepared for the ileal longitudinal muscle by cumulative application of ACH. Treatment with 10\(^{-7}\) M ATR and 10\(^{-6}\) M BASS induced a parallel shift of the dose-response curve to the right (Fig. 3-A). The \(pD_2\) of ACH was 7.97 and the \(pA_2\) of ATR was 8.81. The inhibitory action of BASS on the ACH induced contraction was one order lower than that of ATR. Carbachol and pilocarpine exhibited contractile actions with almost the same properties, and ATR and BASS have an inhibitory actions of essentially the same type. Prolongation of the period of BASS application increases the extent of the inhibition. BASS showed maximal inhibitory effect when applied for 15 min. The inhibitory effect of BASS was almost irreversible.

Histamine (10\(^{-7}\) M) immediately exhibited a tonic contraction. Application of 10\(^{-8}\) M tripelennamine or 10\(^{-6}\) M BASS almost completely inhibited this contraction (Fig. 2-B, B’). Tripelennamine and BASS induced a parallel shift to the right of the dose-response curve obtained by cumulative application of concentrations of histamine beginning with 10\(^{-8}\) M (Fig. 3-B). The muscle began to contract when 10\(^{-8}\) M
Fig. 2. Effect of BASS on the isotonic contraction of ileal longitudinal muscle of guinea pig by various drugs. A: Contraction by ACH and pretreatment with ATR (1 x 10^{-7} M). A': Contraction by ACH and pretreatment with BASS (1 x 10^{-6} M). B: Contraction by histamine (HIS) and pretreatment with tripelennamine (TRIP, 1 x 10^{-8} M). B': Contraction by HIS and pretreatment with BASS (1 x 10^{-5} M). C: Contraction by PGE2 and pretreatment with SC-19220 (3 x 10^{-5} M). C': Contraction by PGE2 and pretreatment with BASS (3 x 10^{-5} M).

Fig. 3. Effect of BASS on the dose response curve of ileal longitudinal muscle to various drugs. Ordinate: Percent contraction relative to the highest contraction in response to 40 mM K. Abscissa: Addition of (A) ACH, (B) HIS (-log M), (C) PGE2 (-log g/ml). All the agents are given in cumulative doses. -log molarity of drug, *: P<0.05 **: P<0.01 relative to control.
|                | pD₂ | Bassianolide | Atropine | Tripelennamine | Morphine  | SC-19220 | Papaverine |
|----------------|-----|--------------|----------|---------------|-----------|----------|------------|
| Acetylcholine  | 7.97| ↓            | ↓*8.81  |               |           |          |            |
| Carbachol      | 7.56| ↓            | ↓*8.73  |               |           |          |            |
| Pilocarpine    | 6.73| ↓            | ↓*8.52  |               |           |          |            |
| Histamine      | 6.70| ↓            |          | ↓*7.10        |           |          |            |
| Serotonin      | 5.98| ↓            |          |               |↓*5.74    |          |            |
| Prostaglandin E₂| 8.20| ↓            |          |               |↓*5.74    |          |            |
| 40 mM KCl      |     | ←           |          |               |↓         |          |            |
| Barium         | 3.43| ←           |          |               |↓         |          |            |

* pA₂
5-HT was applied. The contraction increased in intensity with an increase in concentration of 5-HT, and it reached a maximum after application of about $10^{-6}$ M 5-HT. When a dose-response curve was obtained by single application of 5-HT at each concentration, it showed a gentle sigmoid character. It is known that $10^{-6}$ M morphine and $10^{-6}$ M phenoxybenzamine are antagonistic to M and D receptors of 5-HT (9). Simultaneous pre-application of those two drugs inhibited the contraction caused by 5-HT, and induced a parallel shift to the right of the dose-response curve of 5-HT. Application of $10^{-6}$ M BASS also caused a parallel shift to the right of the dose-response curve of 5-HT.

After application of $10^{-8}$ g/ml PGE2, the contraction appeared immediately and increased in intensity to a maximum. Then the muscle relaxed gradually, accompanied with a spontaneous contraction at relatively high frequency. In this manner, a small and continuous contraction was maintained (Fig. 2-C, C'). The dose-response curve of PGE2 was gently sigmoid (Fig. 3-C). SC-19220 ($3 \times 10^{-5}$ M), which is an antagonist to contraction induced by PGs (14), exhibited an inhibitory action on the contraction by PGE2 (Fig. 2-C, C'). It induced a parallel shift to the right of the dose-response curve of PGE2 (Fig. 3-C). The results obtained from these agonists and antagonists are summarized in Table 1. The inhibitory action of $10^{-6}$ M BASS upon the various agonists mentioned above was almost irreversible.

Application of $10^{-4}$ M Ba caused a contraction about 25% of that induced by 40K. The dose-response curve obtained by cumulative applications of Ba presented a sharp inclination between $10^{-4}$ M and $10^{-3}$ M. The contraction by Ba was inhibited by $2 \times 10^{-6}$ M papaverine, showing changes of a surmountable type. The dose-response curve of $10^{-4}$ to $3 \times 10^{-3}$ M Ba was only slightly changed by application of $10^{-6}$ M BASS. When the high K solution was applied to the medium at a final concentration of 40 or 60 mM, a contraction began immediately after application and reached a maximum in several sec. The contraction induced by 40 or 60 mM K was not significantly inhibited by BASS ($10^{-6}$ to $10^{-5}$ M). Pre-application of papaverine ($1 \times 10^{-6}$ M) inhibited the K contraction.

The vas deferens preparation: Application of $10^{-8}$ to $10^{-7}$ M BASS exerted no influence upon the vas deferens of the guinea pig. When $10^{-5}$ M NE was applied, the muscle immediately exhibited a slight transient contraction followed by a phasic contraction. Pretreatment with $10^{-7}$ M phenolamine inhibited the NE contraction to an extent of about 20%, and with $3 \times 10^{-5}$ M BASS to about 30%. The initial small transient contraction was not influenced at all in either of the cases of pretreatment (Fig. 4-A, A'). Figure 5-A presents the dose-response curve for an application of NE. The pD$_2$ of NE was 5.27. BASS and phenolamine did not inhibit contraction induced by a higher concentration ($1 \times 10^{-4}$ M) of NE (Fig. 5-A). BASS and phenolamine inhibited the contraction induced by phenylephrine, showing a pD$_2$ of 5.10.

Application of $3 \times 10^{-6}$ M ACH gave rise to a two-peak contraction of the vas deferens in the same manner as that with NE. Moreover, $2 \times 10^{-9}$ M ATR or $3 \times 10^{-8}$ M BASS caused 90% inhibition of the phasic contraction induced by ACH. In both cases the initial small transient contraction was not affected (Fig. 4-B, B'). Pretreatment with $2 \times 10^{-9}$ M ATR caused a shift to the right of the dose-response curve of ACH (pD$_2$: 5.57). However, it was difficult to judge whether ATR induced a parallel shift of this curve in the figure or not (Fig. 5-B). Application of $3 \times 10^{-8}$ M BASS inhibited the contraction induced by a lower concentration ($10^{-6}$ to $10^{-5}$ M) of ACH,
but did not inhibit the contraction caused by a higher concentration (3x10^{-5}-10^{-4} M) of ACH. BASS displayed a weaker inhibitory action than ATR. ATR and BASS also inhibited the contraction induced by carbachol, the pD2 of which was 5.50.

When histamine (10^{-4} M) was applied, the vas deferens showed a phasic contraction. In a strip, the contraction due to the first application of histamine for 2 min was 4.36±0.37 g (n=5) and the second application after a 20 min interval decreased to about 70% or 3.28±0.27 g (n=5). The decrease in the tension development by the repeated application of histamine was regarded as a tachyphylaxis. From this reason, antagonists, tripelennamine (10^{-6} M) or BASS (10^{-7} M), were applied 15 min before the second application of histamine. Pretreatment with tripelennamine completely inhibited the contraction induced by the second application of histamine. Application of 10^{-7} M BASS also reduced the second contraction by histamine to about 20% or
0.73±0.4 g (n=5). From these results, tripelennamine and BASS seemes to inhibit the histamine-induced contraction.

The inhibitory action of BASS upon the various agonists mentioned above was almost irreversible.

Application of 3×10⁻³ M Ba induced the phasic contraction of the vas deferens. Papaverine at 3×10⁻⁵ M depressed the Ba-induced contraction, but a small contraction remained which was accompanied by rhythmic contractions. Application of 10⁻⁷ M BASS had no effect on the Ba-induced contraction. Forty mM K induced a phasic contraction followed by a small and tonic one. It was entirely inhibited by 10⁻⁵–3×10⁻⁶ M papaverine, but not influenced at all by 3×10⁻⁸ M BASS. At concentrations ranging from 10⁻⁸ M to 10⁻⁵ M, BASS had no inhibitory effects on the contraction induced by 20, 40 or 60 mM K. As the vas deferens exhibited a poor response to pilocarpine, 5-HT or PGE₂, it was impossible to examine the effect of BASS.

DISCUSSION

When BASS at concentrations from 10⁻⁸ to 10⁻⁵ M was applied to the ileal longitudinal muscle and vas deferens preparations, none of them contracted or relaxed. In the case of intestinal smooth muscle, BASS inhibited the contraction induced by ACH, carbachol and pilocarpine, all of which have an action upon the muscarinic receptor (10, 11). BASS was particularly sensitive to the contraction by ACH. Moreover, BASS inhibited the contractions induced by histamine which interacts with the H₁-receptor (12) and by 5-HT which interacts with M and D receptors (9). On the other hand, it has been reported that the PGE₂-induced contraction is inhibited competitively by SC-19220, and PGE₂ probably acts upon a specific action site (13, 14). BASS also inhibited the contraction induced by PGE₂. The inhibition by BASS showed was a surmountable type and differed from that by SC-19220. However, BASS did not affect the contraction by Ba or high K.

In the vas deferens, BASS inhibited the contraction by NE and phenylephrine which have an action upon α-adrenoceptor (15), the contraction by ACH and carbachol which have an action upon muscarinic receptor (11), and the contraction by histamine which has an action upon the H₁-receptor (12). BASS exerted no influence on the contraction by Ba and high K which seem to act directly upon smooth muscle.

It was found that BASS inhibited all the contractions induced by the drugs which act upon separate receptor systems. The BASS-induced inhibition appears to be non-specific in character, and BASS probably acts at a point in the chain of events leading to contraction, beyond the receptor. However, BASS did not affect the contraction induced by Ba and high K which directly act upon the muscle cell. It is known that Ba or high K increases the free Ca ion level in the cytoplasm of the smooth muscle cell through Ca influx or Ca release from cellular stores, then causes a contraction (16). Accordingly, BASS probably has no influences on the contractile machinery and the Ca movement involved in an increase in free Ca ions in the cytosol. These data suggest that BASS may act upon some steps between the receptor binding and the final common pathway mentioned above.

On the other hand, the ACH receptor has been divided into two sites: (i) the ACH receptor site which binds cholinergic agonists, antagonists (17, 18) and snake venom (α toxins) (19), and (ii) a site involved in the selective permeation of monovalent cations that cause the transient depolarization of the membrane. This site was named the “ACH ionophore” (20) or “ion conductance modulator” (21). Both in vivo (22) and
in vitro (23) local anesthetics, such as prilocaine and tetracaine, affect the permeability response to bath-applied ACH in a manner rather different from that of competitive antagonist like curare. Histrionicoxin (HTX) (24) is an alkaloid isolated from the skin of the Columbian frog, Dendrobates histrionious. HTX showed similar effects in vivo (21, 25, 26) and in vitro (27) to the effects of the local anesthetics on the "ACH ionophore". The local anesthetics, HTX and its derivatives interacted especially with the ACH ionophore or the ion conductance modulator rather than with the ACH recognition site (28–30). These evidence substantially indicate the existence of steps between receptor binding and common contractile processes and suggest the possibility that BASS acts upon the "ACH ionophore" of the receptor. Further study is required to clarify the mode of action of BASS on various kinds of receptors in smooth muscle, including an ACH receptor.

In conclusion, BASS inhibited the contraction induced by the cholinergic drugs, histamine, 5-HT or PGE2 in the ileal longitudinal muscle preparation. It also inhibited the contraction by the adrenergic and cholinergic drugs or histamine in the vas deferens preparation. However, BASS did not affect the contraction by Ba or high K in either of the preparations. These data suggest that BASS inhibits the contractile action of a drug which has an effect upon a selective site of smooth muscle cells, but not the action of a drug which acts upon a non-selective site of smooth muscle cells.

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