MY336-a, A NOVEL β-ADRENERGIC RECEPTOR ANTAGONIST PRODUCED BY *STREPTOMYCES GABONAE*†

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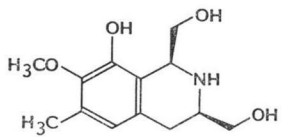
Streptomyces gabonae KY2234 was found to produce a new compound, MY336-a, which bound to β-adrenergic receptor. The compound was isolated from the fermentation broth of KY2234. MY336-a showed a high affinity for the β-receptor, labeled with [^{3}H]dihydroalprenolol in the membrane fractions of rat heart (β_1-adrenergic receptor) or lung (β_2-adrenergic receptor), whereas the compound bound very weakly to α-adrenergic receptor, labeled with [^{3}H]dihydroergokryptine in rat brain. The inhibition constants (*Ki*) of the compound were 0.73 and 0.14 μM for the β-receptors of heart and lung, respectively. 5′-Guanylylimidodiphosphate (Gpp(NH)p) did not alter the affinity of the β-receptors for MY336-a.

In isolated guinea-pig atria, MY336-a produced an inhibition of the positive chronotropic and inotropic effects of isoproterenol. MY336-a also antagonized the relaxation of tone induced by isoproterenol in isolated guinea-pig trachea. No partial agonistic activity was detected in MY336-a in the isolated atria and trachea. In anaesthetized dogs, MY336-a (1 mg/kg, iv) exerted negative inotropic action (left ventricular dp/dt max, −32.6%).

These results indicated that MY336-a is a new antagonist specific for β-adrenergic receptor.

Microbial cells produce a variety of physiologically active compounds. In the course of studies on microbial metabolites, we tried to apply receptor binding assay for seeking pharmacologically active new substances, and found that *Streptomyces gabonae* KY2234 produced a compound with a high affinity for β-adrenergic receptors. The compound, MY336-a, was isolated from the culture broth and its structure was determined to be (1*R**,3*S**)-1,2,3,4-tetrahydro-1,3-bis(hydroxymethyl)-8-hydroxy-6-methyl-7-methoxyisoquinoline (Fig. 1). MY336-a was characterized as a new specific β-adrenergic antagonist, based on the profile of receptor binding assay and pharmacological properties. WILLIAMS *et al.*[1] reported that the culture filtrate of *Bacillus anthracis* exhibited adrenaline-like activity. However, no further data on identification of the active component have been reported so far. This investigation apparently is the first report on the isolation and identification of a microbial metabolite acting on β-adrenergic receptor. In this report, we describe fermentation, isolation

Fig. 1. The structure of MY336-a.



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Abbreviations: DHA; Dihydroalprenolol, DHE; dihydroergokryptine, Gpp(NH)p; 5′-guanylylimidodiphosphate.

and purification, receptor binding properties, and pharmacological activities, of MY336-a. The structure elucidation studies will be described in a separate paper.

Materials and Methods

Microorganisms

S. gabonae KY2234 (ATCC 15282) was employed for the experiments.

Media

The compositions of media for stock, seed, and fermentation cultures were as follows. Stock culture medium (Hickey-Tresner medium); soluble starch 1%, N-Z amine type A 0.2%, beef extract 0.1%, yeast extract 0.1%, and agar 2% (pH 7.2). Seed medium; glucose 1%, soluble starch 1%, beef extract 0.3%, yeast extract 0.5%, Bacto-tryptone (Difco) 0.5%, and $CaCO_3$ 0.2% (pH 7.2 before sterilization). Fermentation medium; dextrin 3%, soy bean meal 2%, corn steep liquor 0.25%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.03%, and $CaCO_3$ (pH 7.8 before sterilization).

Cultures

Four milliliters of a seed medium in a test tube were inoculated with one loopful of organisms grown on a stock culture medium and incubated on a test tube shaker. After incubation for 4 days at 30°C, 1 ml of the seed culture was transferred into a large test tube (2.5 $\phi \times 19$ cm) containing 10 ml of the seed medium. The 2nd seed culture was developed by shaking for 1 day at 30°C and then 20 ml (2 test tube content) of the culture were transferred into 2-liter Erlenmeyer flask containing 400 ml of the same seed medium. After shaking for 2 days at 30°C, 800 ml (2 flask content) of the 3rd seed culture were transferred into 18 liters of the fermentation medium in a 30-liter jar fermentor. The fermentation was conducted for 42 hours at 30°C by stirring at 300 rpm with aeration of 18 liters/minute.

Preparation of Tissue Homogenates

Male Wister rats were killed by decapitation and the hearts and lungs were immediately removed and stocked at −80°C until use. Brains were removed from the skull, and the cerebellum removed and stocked at −80°C until use.

Rat hearts were homogenized with a Brinkmann Polytron PT10 (setting 7, 2 times 30 seconds) in 20 volumes of 50 mM Tris-HCl buffer (pH 8.0) containing 1.0 mM $MgCl_2$. After adding 1 M KCl, the homogenate was filtered through four layers of nylon mesh and centrifuged for 10 minutes at $1,000 \times g$ to remove connective tissue, unbroken cells and cell debris. The supernatant was then centrifuged for 30 minutes at $105,000 \times g$ and the pellets were resuspended in buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 8.0). This washing process was repeated once more before final pellets were resuspended in 10 volumes of assay buffer (50 mM Tris-HCl, pH 7.5). Rat lungs or brains were homogenized with a Brinkmann Polytron PT10 (setting 6~7, 30 seconds) in 20~30 volumes of 50 mM Tris-HCl buffer containing 10 mM $MgCl_2$, pH 8.0 (lung) or 50 mM phosphate buffer, pH 7.0 (brain). The homogenates were centrifuged twice at $50,000 \times g$ for 30 minutes (lung) or 10 minutes (brain), with an intermediate rehomogenization in fresh buffers. The final pellets were resuspended in 10 volumes of 50 mM Tris-HCl buffer, pH 7.6 (lung), or 50 volumes of 50 mM phosphate buffer, pH 7.0 (brain).

Receptor Binding Assay

β_1-Adrenergic Receptor (Heart) and β_2-Adrenergic Receptor (Lung): Tissue (lung or heart) homogenate (13 mg wet weight) was incubated at 25°C in 50 mM Tris-HCl buffer, pH 7.6, with 1.25 nM of [^{3}H]DHA and various additions (final volume, 1.11 ml). After 30 minutes, tissue bound and free ligand were separated by rapid filtration followed by four washes with 4 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.6 on Whatmann fiberglass filter (GF/B). Radioactivity on filter was measured by liquid scintillation spectrometry. [^{3}H]DHA binding in the presence of 9×10^{-6} M (±)-propranolol was defined as non-specific. Binding of [^{3}H]DHA to membrane vesicles from both rat heart and lung demonstrated appropriate stereoselectivity and high affinity for DHA. *Ki*'s obtained from

Scatchard analysis (data not shown) were 5.06 nM and 0.29 nM in heart and lung, respectively. The maximum number of binding sites (Bmax) for DHA was approximately 8.4 pmol/g tissue (wet weight) in heart and 26 pmol/g tissue (wet weight) in lung. Inhibition constants (*Ki*) for MY336-a and various drugs were calculated from the equation $Ki=IC_{50}/(1+S/K_D)$,[2] where IC_{50} was the concentration to produce 50% displacement, and S is the concentration of [^{3}H]DHA.

α-Adrenergic Receptor: Brain homogenate (22 mg wet weight) were incubated at 25°C in 50 mM phosphate buffer, pH 7.0, with 0.5 nM [^{3}H]DHE and various additions (final volume 2.1 ml). After 45 minutes, the incubation mixture were filtered rapidly through Whatmann GF/B filter. The filters were washed five times with 3 ml of ice-cold 50 mM phosphate buffer, pH 7.0, and were subsequently counted by liquid scintillation spectrometry. [^{3}H]DHE binding in the presence of 1×10^{-4} M noradrenaline was defined as non-specific. Binding of [^{3}H]DHE to the brain membrane vesicles from rat demonstrated appropriate stereoselectivity and high affinity for DHE. From Scatchard analysis (data not shown), *Ki* and Bmax were calculated to be 2.0 nM and 11.5 pmol/g tissue (wet weight), respectively.

Pharmacological Method

Antagonism of MY336-a against isoproterenol stimulated β_1- and β_2-adreno-receptors was examined in the guinea-pig isolated atria and tracheae, respectively.

Atria were rapidly isolated from guinea-pigs (body weight 500~700 g) and mounted in an organ bath filled with 30 ml of oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution at a temperature of 31°C. Atria were allowed to beat spontaneously, and the contractions were isometrically recorded using force-displacement transducer. Resting force was adjusted to 0.5 g.

Tracheae were isolated from guinea-pigs (body weight 500~700 g), cut zig-zag, and mounted in an organ bath. The bath was filled with 30 ml oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution at a temperature of 37°C. Resting force was adjusted to 0.5 g. Tracheae were contracted by acetylcholine hydrochloride (20 μg/ml), and the contractions were isometrically recorded using force-displacement transducer.

In vivo pharmacological activity of MY336-a as a β-blocking agent was examined as follows. A male mongrel dog, weighing 8 kg, was anaesthetized with sodium pentobarbital, 30 mg/kg, iv. An intratracheal cannula was inserted, and artificial ventilation was carried out using room air. The forelimb vein was cannulated and used for intravenous administration of drugs. A right femoral artery was cannulated for monitoring blood pressure. A lead II ECG was monitored by attaching pin electrodes. The left ventricular pressure was measured with a Millar Mikro-Tip catheter transducer inserted into left ventricle. The dp/dt max was calculated, using Hitachi mini-computer.

Materials

[^{3}H]DHA (72~102 Ci/mmol) and [^{3}H]DHE (20 Ci/mmol) were purchased from Amersham and New England Nuclear, respectively. (±)-Propranolol, (−)-alprenolol, (−)-adrenaline, and (−)-isoproterenol were from Sigma and (−)-noradrenaline was from Nakarai Chemicals Ltd.

Results

Fermentation

Culture filtrate of *S. gabonae* KY2234 exhibited an inhibitory activity against specific binding of [^{3}H]DHA in rat heart. A time course of the fermentation with 30-liter jar fermentor is shown in Fig. 2. The inhibitory activity increased rapidly between 20 hours and 30 hours, and then reached plateau, accompanying rise of pH.

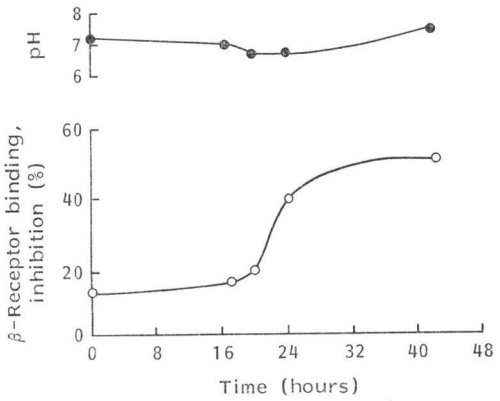
Isolation and Purification of MY336-a

The procedure for isolation of MY336-a is shown schematically in Fig. 3. Fermentation broth (36 liters) was adjusted to pH 4~5 with hydrogen chloride and centrifuged with a Sharples centrifugator.

Fig. 2. A time course of MY336-a production.

S. gabonae KY2234 was cultured in 30-liter jar fermentor containing the production medium.

β-Adrenergic receptor binding activity was measured as described under "Materials and Methods", where 100 μl of broth filtrate was added in the reaction mixture.



The supernatant was applied to a Diaion HP-10 resin column (2 liters) and eluted with 10 liters of 50% methanol. The eluate was evaporated under reduced pressure to a volume of 3 liters, adjusted to pH 5.0 with hydrogen chloride, and then applied to 2 liters of a Diaion SK 1B resin (NH_4^+) column. The resin was washed with distilled water and the active components were eluted with 6 liters of 1 N ammonium hydroxide. The eluate was evaporated under reduced pressure to a volume of 2 liters, adjusted to pH 7.0 with hydrogen chloride, and then applied to 600 ml of CM-Sephadex C-25. After washing with 1.8 liters of 0.01 M phosphate buffer (pH 8.0), elution was performed with 1.8 liters of 0.05 M phosphate buffer (pH 8.0). The eluate was applied to 300 ml of Diaion SK 1B resin (NH_4^+), and eluted with 900 ml of 1 N ammonium hydroxide. The eluate was evaporated to a volume of 100 ml, adjusted to pH 7.0 with hydrogen chloride, and then chromatographed on CM-Sephadex

Fig. 3. Isolation of MY336-a.

Fermentation broth (36 liters)
- adjusted to pH 4
- centrifuged

Supernatant

Diaion HP-10
- eluted with 50% MeOH
- evaporated

Diaion SK 1B (NH_4^+)
- eluted with 1 N NH_4OH
- evaporated

CM-Sephadex C-25
- washed with 0.01 M phosphate buffer, pH 8.0
- eluted with 0.05 M phosphate buffer, pH 8.0
- desalted with Diaion SK 1B (NH_4^+)

CM-Sephadex C-25
- eluted with a linear gradient of 0~0.05 M phosphate buffer, pH 8.0
- desalted with Diaion SK 1B (NH_4^+)

Crude MY336-a

Silica gel column chromatography
- BuOH - EtOH - $CHCl_3$ - conc NH_4OH (4: 5: 2: 2)

Fractions containing MY336-a
- evaporated to dryness
- crystallized from MeOH

Crystalline MY336-a

C-25 (400 ml). Elution was performed with a linear gradient between 2 liters of water and 2 liters of 0.05 M phosphate buffer, pH 8.0. Two hundred fractions (20 ml each) were collected. Five peaks of inhibitory activity could be detected by the binding assay to the β-receptor of heart. Fractions 150~173, which contained a major iodine-positive substance (MY336-a) detected on a silica gel thin-layer chromatogram, were combined, desalted with Diaion SK 1B (NH_4^+) and evaporated to dryness. The crude powder of MY336-a was further chromatographed on a silica gel (Wako gel C-200) column. The column was developed with butanol - ethanol - chloroform - conc ammonia (4: 5: 2: 2). The fractions containing MY336-a were collected, evaporated to dryness, and dissolved with a small amount of methanol. The solution was kept standing at 4°C for 2 or 3 days to crystallize MY336-a (35 mg).

MY336-a thus obtained was colorless, needle-like crystal, with melting point 177~178°C. The compound was readily soluble in acidic water, soluble in water and methanol, and sparingly soluble in acetone, hexane and ether. The Rf values of the compound on silica gel thin-layer chromatogram are 0.02, 0.29, and 0.43 in the solvent system of chloroform - methanol (9: 1), propanol - ethyl acetate - water (1: 1: 1), and acetic acid - chloroform - methanol (1: 7: 2), respectively. The structure of MY336-a was determined to be (1*R**,3*S**)-1,2,3,4-tetrahydro-1,3-bis(hydroxymethyl)-8-hydroxy-6-methyl-7-methoxyisoquinoline (Fig. 1) on the basis of MS, NMR and X-ray crystallographic data. The detail studies will be published in a separate paper.

Binding of MY336-a to Adrenergic Receptors in Rat Heart, Lung and Brain

Binding to β-Adrenergic Receptors in Rat Heart (β_1) and Lung (β_2)

The effect of MY336-a on specific [^{3}H]DHA binding was examined in rat heart and lung, comparing with various β_1- or β_2-adrenergic antagonists and agonists. Figs. 4 and 5 illustrated the ability of MY336-a and adrenergic agonists (adrenaline, noradrenaline, isoproterenol) and antagonist (propranolol) to compete for DHA binding to the rat heart and lung. From the IC_{50} obtained from the displacement curve, *Ki*'s were calculated as described in the section, "Materials and Methods", and listed in Table 1. MY336-a inhibited [^{3}H]DHA binding to both heart and lung in a dose dependent

Fig. 4. Inhibition of [^{3}H]DHA binding to β-adrenergic receptor in rat heart by MY336-a (○), (±)-propranolol (●), (−)-isoproterenol (△), and (−)-adrenaline (▲).
The ordinate indicates the percent of maximal specific DHA binding.
Values are means from triplicate experiments.

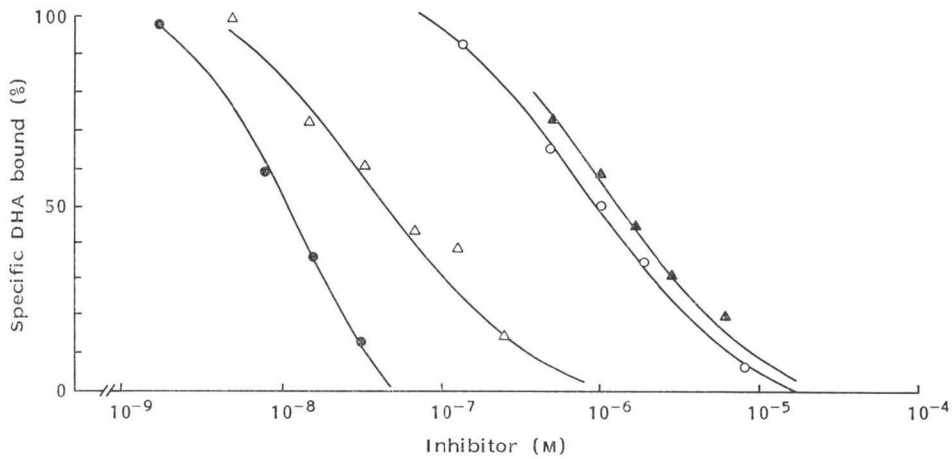


Fig. 5. Inhibition of [^{3}H]DHA binding to β-adrenergic receptor in rat lung by MY336-a (○), (±)-propranolol (●), (−)-isoproterenol (△), (−)-adrenaline (▲), and (−)-noradrenaline (□).
The ordinate indicates the percent of maximal specific DHA binding.
Values are means from triplicate experiments.

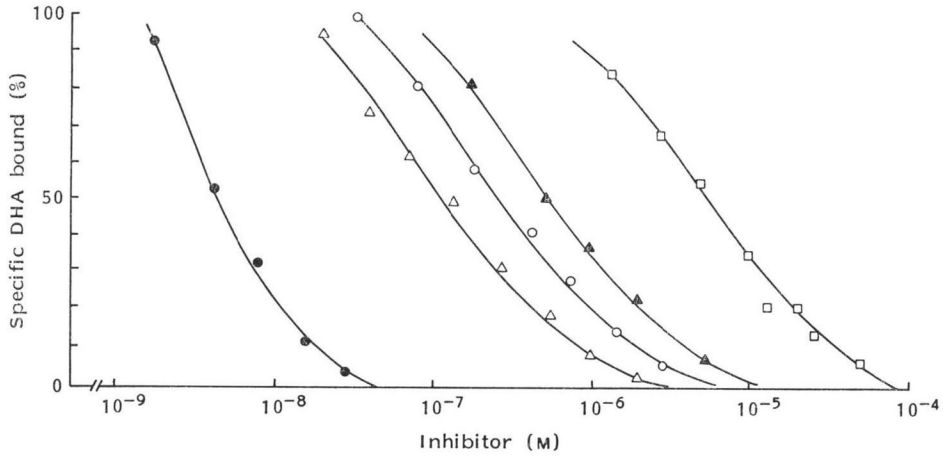
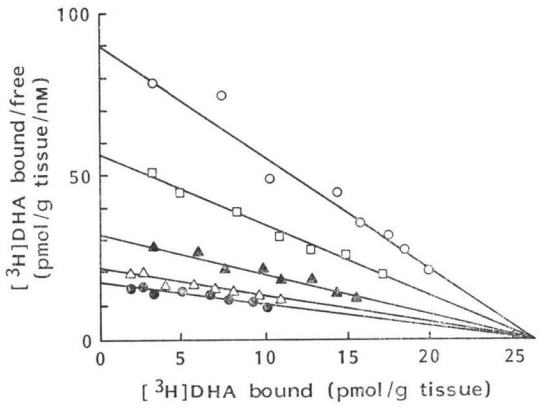


Table 1. *Ki* values of MY336-a and various β-adrenergic agonists and antagonists.

Drug	*Ki* (μM)	
	β-Adrenergic receptor in rat atria	β-Adrenergic receptor in rat lung
(−)-Adrenaline	0.821	0.317
(−)-Noradrenaline	0.497	3.346
(−)-Isoproterenol	0.017	0.067
(−)-Alprenorol	0.0046	0.0011
(±)-Propranolol	0.0073	0.0015
MY336-a	0.730	0.140

Fig. 6. Scatchard analysis of the effects of MY336-a on [^{3}H]DHA binding in rat lung.
Equilibrium measurements of [^{3}H]DHA binding was made at various concentrations of [^{3}H]ligand in the absence (○) or presence of 1.07×10^{-7} M (□), 1.78×10^{-7} M (▲), 3.53×10^{-7} M (△), and 7.13×10^{-7} M (●) of MY336-a.
Values are means from triplicate experiments and data are representative of three such experiments made with different membrane preparations.



manner. *Ki* values for MY336-a were 0.14 μM in lung and 0.73 μM in heart. The efficacy of MY336-a in inhibiting the binding of [^{3}H]DHA was comparable to (−)-noradrenaline (*Ki*, 0.497 μM) in heart, and half of (−)-isoproterenol (*Ki*, 0.067 μM) in lung.

The inhibition of specific [^{3}H]DHA binding in lung (β_2) by MY336-a was measured at different concentrations of [^{3}H]DHA. Scatchard plots at different concentrations of MY336-a were linear, and the various straight lines intercept each other at one single point located on the abscissa axis (Fig. 6). This indicates that the displacement is characteristic of competitive inhibitor. Similar Scatchard analysis in cardiac β-adrenergic receptor (β_1) binding indicated that MY336-a inhibited [^{3}H]DHA binding in competitive manner (data not shown). Thus MY336-a is a competitive inhibitor of β_1- and β_2-adrenergic receptor binding.

In the experiments depicted in Fig. 7, the specific [^{3}H]DHA binding in lung was measured in the

Fig. 7. Effect of Gpp(NH)p on competition of the agonist (−)-isoproterenol (□, ■), the antagonist (±)-propranolol (△, ▲) and MY336-a (○, ●) for [^{3}H]DHA binding sites in rat lung membrane.

Rat lung membranes (13 mg wet weight) were incubated at 25°C in 50 mM Tris-HCl buffer, pH 7.6, with 1.25 nM of [^{3}H]DHA and each of inhibitors in the presence (closed symbols) or absence (open symbols) of 50 μM Gpp(NH)p.

Binding activity was measured as described under "Materials and Methods".

The ordinate indicates the percent of maximal specific DHA binding.

Values are means from triplicate experiments and the data are representative of three such experiments made with different membrane preparations.

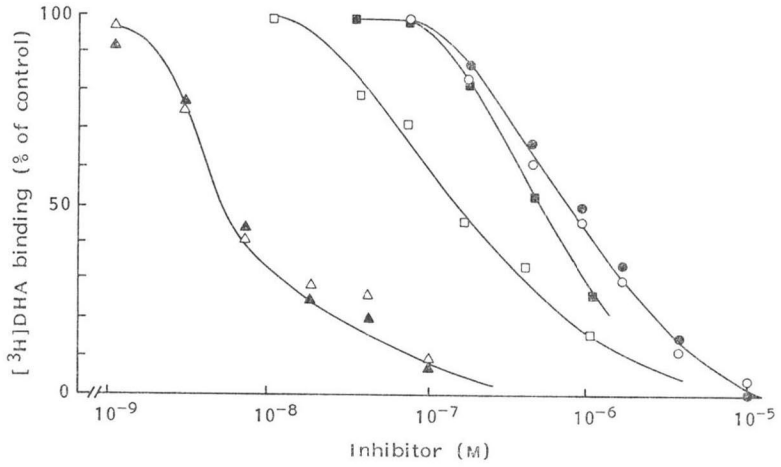
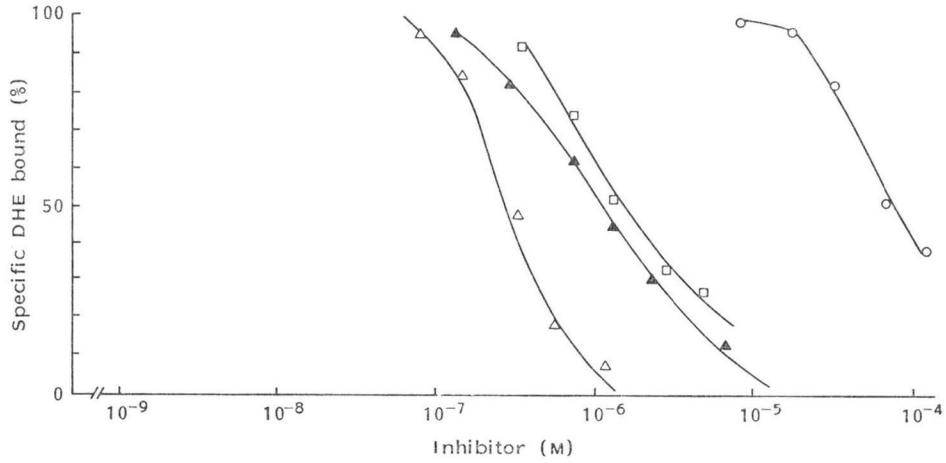


Fig. 8. Inhibition of [^{3}H]DHE binding to α-adrenergic receptor in rat brain by MY336-a (○), phenoxybenzamine (△), (−)-adrenaline (▲), and (−)-noradrenaline (□).

The ordinate indicates the percent of maximal specific DHE binding.

Values are means from triplicate experiments.



presence of MY336-a and β-adrenergic agonist (isoproterenol) and antagonist (propranolol) together with Gpp(NH)p. Gpp(NH)p has been reported to decrease the affinity of β-adrenergic agonists to the β-receptor.[3] In contrast, the affinity of β-adrenergic antagonists was not affected by Gpp(NH)p. Actually, the affinity of isoproterenol was decreased by the addition of Gpp(NH)p (50 μM), whereas Gpp(NH)p showed no effect on propranolol binding. The affinity of MY336-a was not changed by

Fig. 9. β_1-Antagonistic effect of MY336-a in isolated guinea-pig atria.

Isolated guinea-pig atria were allowed to beat spontaneously in Krebs-Henseleit solution, and contractions were isometrically recorded using force-displacement transducers (see "Materials and Methods").

a) Isoproterenol hydrochloride (ISO) (1 ng/ml) induced increase of atrial rate and atrial contractile force.

b) MY336-a (final conc 30 μg/ml) was added to Krebs-Henseleit solution 30 minutes before adding ISO (1 ng/ml).

c) After removing the agents, ISO (1 ng/ml) was added again.

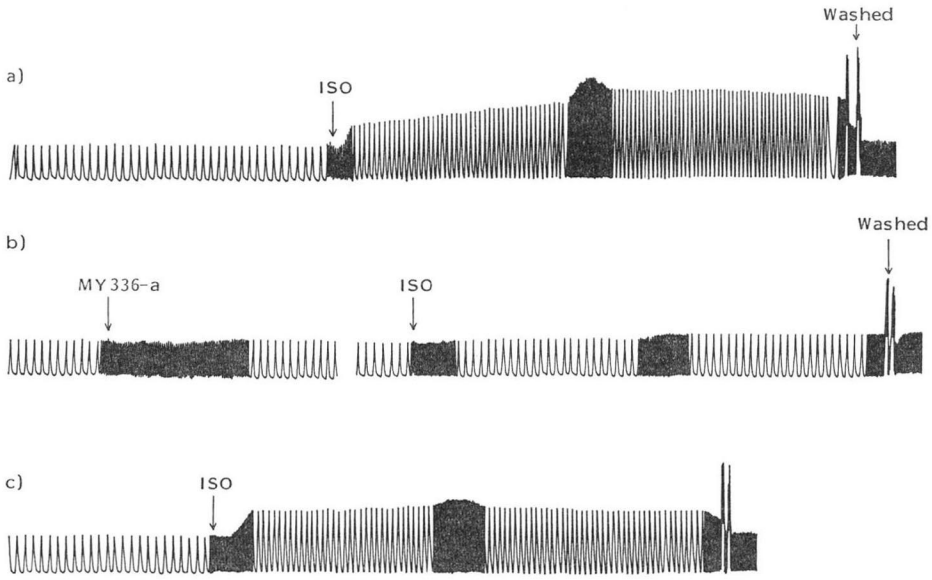
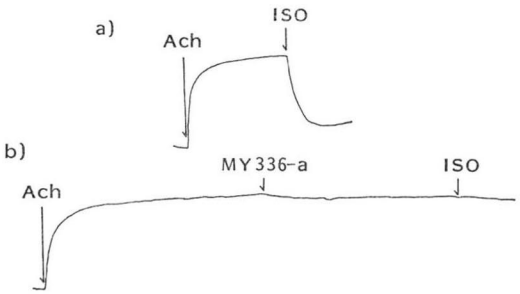


Fig. 10. β_2-Antagonistic effect of MY336-a in isolated guinea-pig tracheae.

Isolated guinea-pig trachea was mounted in an organ bath containing Krebs-Henseleit solution and the contractile force were isometrically recorded using force-displacement transducer.

a) Trachea was contracted by acetylcholine hydrochloride (Ach) (20 μg/ml). Isoproterenol hydrochloride (ISO) (0.2 μg/ml) induced the relaxation of tone.

b) MY336-a (30 μg/ml) was added to the organ bath before treatment of ISO (0.2 μg/ml).



the addition of Gpp(NH)p. These results suggested that MY336-a was a β_2-adrenergic antagonist. Similar antagonistic properties were observed in cardiac β_1-adrenergic receptor binding (data not shown).

Binding to α-Adrenergic Receptors in Rat Brain

The effects of MY336-a on specific [^{3}H]DHE binding were examined in rat brain. As shown in Fig. 8, MY336-a weakly inhibited the binding of [^{3}H]DHE; the IC_{50} was around 10^{-4} M, two or three orders magnitude less potent than those for β-adrenergic receptors.

Pharmacological Properties of MY336-a

Preliminary pharmacological studies were undertaken to characterize MY336-a.

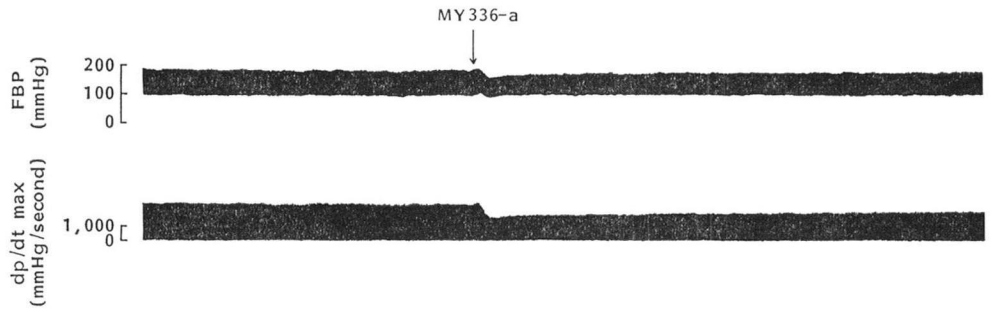
In isolated guinea-pig atria, 30 μg/ml of MY336-a antagonized against the isoproterenol (1 ng/ml)-induced increase of atrial rate and atrial

Fig. 11. Negative inotropic effect of MY336-a in normal anaesthetized dogs.

MY336-a (30 μg/ml) was administrated intravenously to the fore limb vein of a mongrel dog anaesthetized with sodium pentobarbital.

A right femoral artery was cannulated for monitoring blood pressure (FBP).

The left ventricular pressure was measured with a Millar Mikro-Tip catheter transducer inserted into left ventricle. The dp/dt max was calculated, using Hitachi mini-computer (see "Materials and Methods").



contractile force up to 80% (Fig. 9). The data indicate that MY336-a possesses β_1-antagonistic activity.

In isolated guinea-pig trachea, MY336-a, 30 μg/ml, antagonized the relaxation of tone induced by isoproterenol hydrochloride (0.2 μg/ml) up to 87% ($n=3$) (Fig. 10), indicating that MY336-a had β_2-antagonistic activity. No partial agonistic activity was detected in MY336-a in the isolated atria and trachea (data not shown).

In normal anaesthetized dogs, intravenous administration of MY336-a, 1 mg/kg, excerted negative inotropic effect; left ventricular dp/dt max (a parameter of myocardial contractile force) was -32.6% without significant change of blood pressure (Fig. 11).

Discussion

Many of microbial metabolites have been found to possess pharmacological activity.[4] Some of the compounds were found to have pharmacological properties secondary to their antibiotic properties. Seeking enzyme inhibitors has been provided an efficient method for screening new pharmacologically active substances from microbial metabolites. Receptor binding assay has been developed as a simple technique used with a wide range of receptor sites and provided useful probes for drug development programs.[5] In this investigation, we attempted to apply the receptor binding assay technique for seeking new pharmacologically active substances from microbial metabolites. A fermentation broth of *S. gabonae* KY2234 was found active in a β-adrenergic receptor binding assay. Monitoring the active components by the receptor binding assay, the main component was purified from the culture broth. The purified compound, MY336-a, had a novel tetrahydroisoquinoline structure and showed high affinities for the β_1- and β_2-adrenergic receptors. Furthermore, MY336-a was proved to be pharmacologically active both *in vitro* and *in vivo*; the compound inhibited the positive chronotropic and inotropic effects of isoproterenol in isolated atria preparation. Isoproterenol-induced bronchodilation was antagonized by MY336-a. In normal anaesthetized dogs, MY336-a excerted negative inotropic action. These results indicate that MY336-a is β_1- and β_2-adrenergic antagonist. Thus, it was demonstrated that the receptor binding assay was very useful to find new pharmacologically active substances from microbial metabolites.

The binding activity of MY336-a for α-adrenergic receptor in rat brain was two or three orders of magnitude weaker than those for the β-adrenergic receptors. MY336-a hardly bound to opiate receptors, labeled with [^{3}H]naloxon in rat brain or to dopamine and serotonin receptors, labeled with [^{3}H]spiperone in rat striatum (data not shown). These results indicate that MY336-a is a specific

antagonist for β-adrenergic receptors. The affinity of MY336-a for the β-adrenergic receptor of rat lung was approx. Six-fold higher than that of rat heart. MINNEMAN *et al.*[6] reported that the calculated ratio of β_1: β_2-adrenergic receptors were; rat heart 83: 13 and rat lung 15: 85. Therefore, the binding assay using rat heart and lung could measure dominantly β_1- and β_2-adrenergic receptors, respectively. The displacement profiles of adrenergic agents for each receptor (Figs. 4 and 5, Table 1) agreed with the tissue specificity of β-adrenergic receptors. Therefore, MY336-a showed higher affinity for β_2-receptor than that for β_1-receptor. However, the compound proved to be nonspecific β_1- and β_2-adrenergic antagonist in pharmacological activities.

MY336-a is a novel compound belonging to simple isoquinoline alkaloids.[7] Although a vast number of isoquinoline alkaloids in plant have been reported, the hydroxymethyl substituents at C-1 and C-3 positions and 6-methyl substituent are uncommon.[7] Only calycotamine and pterocereine, both are cactus alkaloids, have a hydroxymethyl substituent at C-1 position. The pharmacological effects of simple isoquinoline alkaloids and similar compounds have been the subject of a number of reports in the literature. However, none of the naturally occurring compounds appeared to possess activities interesting enough for more elaborate use as a pharmacological tool or as drug. Some isoquinoline alkaloids have been shown to possess agonistic or antagonistic activity on adrenergic receptors. One of the most potent compound, tetrahydropapaveloline (THP) (the condensation product of dopamine and 3,4-dihydroxyphenylacetaldehyde), has been reported to act as antagonist of [^{3}H]DHA binding to β-adrenergic receptors.[8] Comparing with THP (IC_{50}; 3.5 and 1.8 μM for β_1- and β_2-adrenergic receptors, respectively), MY336-a was one order magnitude more potent.

The β-blocking agents in therapeutic use are either arylethanolamines or (aryloxy)-propanolamines. Only few examples of tetrahydroisoquinolines as β-blocking agents have been described. Pharmacological studies of MY336-a have been preliminary yet and more precise pharmacological and toxicological studies will be required for development of MY336-a as a drug. Moreover, it may be worthwhile investigating the chemical modification of MY336-a in order to increase β-blocking activity or studying structure-activity relationship in the derivatives from this unique lead compound.

In conclusion, we found a novel compound, MY336-a, with a potent and specific β-blocking activity from the culture broth of *S. gabonae*, utilizing receptor binding assay technique. Receptor binding assay will be widely and efficiently applicable for screening of new pharmacologically active compounds from microbial origin.

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