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Biochemical Pharmacology 67 (2004) 815–822
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Characterization of mexiletine as an antagonist of β-adrenoceptor in Chinese hamster ovary cells expressing cloned human β-adrenoceptors

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Received 3 July 2003; accepted 26 September 2003

Abstract

We characterized the β-adrenoceptor-blocking property of mexiletine, a class Ib antiarrhythmic drug, on Chinese hamster ovary (CHO) cells stably expressing cloned human β_1-, β_2-, and β_3-adrenoceptors. In radioligand binding experiments, mexiletine (10 μM–1 mM) concentration-dependently displaced the specific binding of [^{125}I]cyanopindolol to human β_1- and β_2-adrenoceptors in the membrane fraction of the cells. High concentration (100 μM–1 mM) of mexiletine partially displaced the specific binding of [^{125}I]cyanopindolol to human β_3-adrenoceptor. On the other hand, high concentration (300 μM and 1 mM) of lidocaine, another class Ib antiarrhythmic drug, partially displaced the specific binding of [^{125}I]cyanopindolol to human β_1-adrenoceptor, whereas it did not affect the specific binding of [^{125}I]cyanopindolol to human β_2- and β_3-adrenoceptors. Mexiletine (5, 50, and 500 μM) reduces basal adenosine 3′,5′-cyclic monophosphate (cAMP) level and isoprenaline-induced cAMP accumulation on CHO cells stably expressing cloned human β_1- and β_2-adrenoceptors. Lidocaine (10 and 100 μM and 1 mM) tend to reduce basal cAMP level on CHO cells stably expressing cloned human β_1-adrenoceptors, whereas the drug did not reduce the isoprenaline-induced cAMP accumulation on CHO cells stably expressing cloned human β_1-, β_2-, and β_3-adrenoceptors. Mexiletine and lidocaine have no effect on forskolin (0.1, 1, and 3 μM)-induced cAMP accumulation. These results demonstrate that mexiletine blocks the binding of agonists to β_1- and β_2-adrenoceptors, and thereby attenuates the agonist-induced cAMP accumulation, and that the action of mexiletine as an antagonist of β_1- and β_2-adrenoceptors is independent of its antiarrhythmic property.

Keywords: Adenosine 3′,5′-cyclic monophosphate; G protein-coupled receptor; β-Adrenoceptor; Class Ib antiarrhythmic drug; Lidocaine; Mexiletine

1. Introduction

Class Ib antiarrhythmic drugs, such as mexiletine and lidocaine, have been used to treat ventricular arrhythmias. They reduce Na^+ currents, resulting in the inhibition of depolarization of cell membranes. In addition, they have the inhibitory effect on Ca^{2+} channels. Thus, they relax various smooth muscle preparations [1,2]. β-Adrenoceptor antagonists have been used to treat various cardiovascular diseases, such as hypertensions, angina of effort, and ventricular arrhythmias. The β-antagonists which have a membrane-stabilizing effect, for example, propranolol, are known to reduce Na^+ currents weakly. Although β-antagonists do not affect smooth muscle generally, they contract tracheal smooth muscle because trachea is under a strong sympathetic control.

Our recent study demonstrated that mexiletine inhibits the binding of β_2-agonists to their receptors, and attenuates β_2-agonist-induced relaxation and cAMP accumulation on the bovine tracheal smooth muscle [3]. We also showed that lidocaine augmented relaxant responses to cAMP-elevating agents, such as salbutamol and forskolin, through enhancement of cAMP accumulation in the bovine airway smooth muscle [4]. In contrast, lidocaine was reported to inhibit the binding of ligands to β_2-adrenoceptors and attenuate agonist-induced cAMP accumulation on human lymphocytes [5,6]. These data suggest that mexiletine and/or lidocaine affect the pharmacological effect of β-adrenoceptors in some ways that relating to production of cAMP. However, whether mexiletine and lidocaine affect the binding of ligands to cloned human β_1-, β_2-, and β_3-adrenoceptors remains to be elucidated.

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Abbreviations: CHO, Chinese hamster ovary; cAMP, adenosine 3′, 5′-cyclic monophosphate.

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doi:10.1016/j.bcp.2003.09.044

The aim of the present study was to examine β-adrenoceptor-blocking property of mexiletine and lidocaine on CHO cells stably expressing cloned human β_1-, β_2-, and β_3-adrenoceptors. For this purpose, we evaluated the effects of mexiletine and lidocaine on the binding of [^{125}I]cyanopindolol to human β_1-, β_2-, and β_3-adrenoceptors and the isoprenaline-induced changes in the intracellular cAMP content in CHO cells stably expressing human β_1-, β_2-, and β_3-adrenoceptors.

2. Materials and methods

2.1. CHO-K1 cells stably expressed β-adrenoceptors

CHO-K1 cells expressed the β_1- and β_2-adrenoceptors with HA tag recognized by monoclonal antibody 12CA5 (sequence YPYDVPDYA) at the N-terminus were grown as monolayer in Ham's F-12 supplemented with 10% fetal bovine serum and 300 mg/L G418 sulfate in atmosphere of 95% air and 5% CO_2 at 37°. According to Sato *et al*. [7], the HA-tagged β_1- and β_2-adrenoceptors were expressed more stably than the wild types. The cDNA encoding human β_3-adrenoceptors was inserted into pcDNA3 expression vector. The pcDNA3 expression vector containing the cDNA encoding human β_3-adrenoceptor was transfected into CHO-K1 cells using TransFast^{TM} reagent. The stable transformants were selected in Ham's F-12 supplemented with 10% fetal bovine serum and 1 g/L G418 sulfate in atmosphere of 95% air and 5% CO_2 at 37°. The transfected cells were grown as monolayer in Ham's F-12 supplemented with 10% fetal bovine serum and 300 mg/L G418 sulfate in atmosphere of 95% air and 5% CO_2 at 37°. Expression of β_1-, β_2-, and β_3-adrenoceptors was determined by radioligand receptor binding assay using [^{125}I]cyanopindolol as described below.

2.2. Membrane preparation

The CHO-K1 cells expressing the β-adrenoceptors were washed three times with ice-cold PBS and scraped in 1 mL of lysis buffer, comprising of 10 mM Tris–HCl (pH 7.4), 5 mM EDTA, 5 mM EGTA, 2.5 μg/mL pepstatin A, 0.1 mM phenylmethylsulfonylfluoride, and 1 μM leupeptin. The cells were homogenized with ULTRA-TURRAX homogenizer (model T25-BS4, IKA Labortechnik) and centrifuged at 45,000 *g* for 30 min at 4°. The supernatant was discarded and the pellet was resuspended in 10 mM HEPES–NaOH (pH 7.4). The resuspended membrane was frozen with liquid nitrogen and stocked at −80° until use.

2.3. Radioligand receptor binding assay

Radioligand receptor binding studies were carried out in reaction buffer containing 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$ at 37° for 60 min using 50 μg membrane protein. The total reaction volume was 300 μL. For saturation isotherms, membranes were incubated with various concentrations of [^{125}I]cyanopindolol (3.75–120 pM for β_1 and β_2, 150–3000 pM for β_3) in the absence or presence of 10 μM (±)-propranolol for β_1- and β_2-adrenoceptors or 1 mM (±)-propranolol for β_3-adrenoceptors. Competition binding studies were carried out using 120 pM (for β_1 and β_2) or 1700 pM (for β_3) [^{125}I]cyanopindolol and various concentrations of mexiletine and lidocaine (0–1000 μM). The reaction was stopped by 5 mL of ice-cold reaction buffer, followed by rapid filtration over Whatman GF/C filters (Whatman) using a cell harvester (model M-12, Brandel). The radioactivity remaining on the filter was counted by a gamma counter (ARC-300, Aloka).

Kinetic experiments were carried out with 120 pM [^{125}I]cyanopindolol for β_1- and β_2-adrenergic receptors. Association initiated by addition of membranes and dissociation by addition of 10 μM propranolol after 60 min of incubation with [^{125}I]cyanopindolol. Reaction was stopped at point of time between 30 s and 60 min.

2.4. Measurement of cyclic AMP accumulation

The CHO-K1 cells expressed of β_1-, β_2-, and β_3-adrenoceptors were grown as a monolayer on 6-well culture plates in Ham's F-12 medium with 10% fetal bovine serum and 300 mg/L G418 sulfate. The medium was replaced with modified Krebs–Henseleit buffer solution, comprising of 119 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose, 0.5 mM 3-isobutyl-1-metylxanthine, and 1 mM ascorbic acid, and incubated in atmosphere of 95% air and 5% CO_2 at 37°. After 30 min, various concentrations of mexiletine (0–500 μM) and lidocaine (0–1000 μM) were added and incubated for 15 min. Then, the CHO-K1 cells were stimulated by (−)-isoproterenol (0–10 μM) and forskolin (0.1–1 μM) for 5 min. The reaction was terminated by addition of 30% (w/v) trichloroacetic acid and the cells were scraped. The samples were sonicated for 5 min and centrifuged at 1500 *g* for 10 min at 4°. Cyclic AMP containing the supernatant was extracted by water-saturated diethyl ether, and measured by radioimmunoassay using cAMP kit "YAMASA" [8].

2.5. Protein assay

Protein contents were measured by the method of Lowry *et al*. [9]. BSA was used as the standard.

2.6. Data analysis and statistics

Data from radioligand saturation assays, competition curves, and kinetic experiments were fitted by nonlinear least-squares analysis using a computer program, GraphPad Prism (GraphPad software). The equilibrium dissociation constant (K_d) and the maximum binding capacity

(B_{max}) of [^{125}I]cyanopindolol were calculated from Scatchard analysis. The affinity of receptors for mexiletine and lidocaine in competition for radioligand binding (K_i) was determined by the methods of Cheng and Prusoff [10]. All results were expressed as means ± SEM. One-way ANOVA followed by Dunnet's test was used for multiple comparisons. Differences were considered to be statistically significant when the P value was less than 0.05.

2.7. Materials

The cDNA encoding β_1- and β_2-adrenoceptors were kindly provided by Dr. R.J. Lefcowitz (Duke University, Durham, NC, USA). CHO-K1 cells expressing the β_1- and β_2-adrenoceptors and the cDNA encoding β_3-adrenoceptors were kindly provided by Dr. Hitoshi Kurose and Dr. Taku Nagao (University of Tokyo, Tokyo, Japan). [^{125}I]Cyanopindolol (2000 Ci/mM, Amersham-Pharmacia Biotech), cAMP kit "YAMASA" (Yamasa Shoyu), (−)-isoproterenol (Sigma), mexiletine (Sigma), (±)-propranolol (Sigma), lidocaine (Sigma), forskolin (Sigma), Ham's F-12 medium (Life Technologies), fetal bovine serum (Life Technologies), G418 sulfate (Nakalai Tesque), pcDNA3 expression vector (Invitrogen), and other chemicals were purchased from standard commercial sources.

3. Results

3.1. Binding properties of mexiletine and lidocaine to β-adrenoceptor subtypes expressed on CHO-K1 cell membranes

Table 1 shows the binding properties of [^{125}I]cyanopindolol to the membrane fraction prepared from CHO-K1 cells expressing one of the β-adrenoceptor subtypes. Specific binding of [^{125}I]cyanopindolol to each of the β-adrenoceptors was monophasic and saturable, indicating a single binding site. Expression of β_3-adrenoceptors was much higher as compared with those of β_1- and β_2-adrenoceptors. The binding affinity of [^{125}I]cyanopindolol to the β_3-adrenoceptors was low, as reported previously [11]. In the present study, we calculated for the fit of all displacements of [^{125}I]cyanopindolol using both monophasic and biphasic algorithms. Monophasic, but not biphasic, curves fitted the best in all of the displacements by nonlinear least-squares analysis using a computer program, GraphPad Prism. Table 2 shows the properties of competition of [^{125}I]cyanopindolol binding with mexiletine and lidocaine in the membrane fraction prepared from CHO-K1 cells expressing one of the β-adrenoceptor subtypes. Mexiletine caused monophasic displacement of [^{125}I]cyanopindolol bound to human β_1-, β_2-, and β_3-adrenoceptors, suggesting a single binding site (Fig. 1). The slope factor for β_1-adrenoceptors is 0.54 ± 0.07, that for β_2-adrenoceptors is 0.66 ± 0.05, and that for β_3-adrenoceptors is 0.47 ± 0.08. One millimole per litre mexiletine displaced approximately 80% of the specific binding of [^{125}I]cyanopindolol to β_1- and β_2-adrenoceptors and 50% of that to β_3-adrenoceptors. The affinity of mexiletine for β_1-adrenoceptors was almost the same as that for β_2-adrenoceptors, and approximately five times higher than that for β_3-adrenoceptors. In contrast to mexiletine, lidocaine caused monophasic displacement of [^{125}I]cyanopindolol only bound to β_1-adrenoceptors (Fig. 2). One millimole per litre lidocaine displaced approximately 40% of the specific binding of [^{125}I]cyanopindolol to human β_1-adrenoceptors. The affinity of lidocaine for β_1-adrenoceptors was approximately 17 times lower than that of mexiletine.

Table 1
Binding properties of [^{125}I]cyanopindolol to the membrane fraction prepared from Chinese hamster ovary cells expressing human β-adrenoceptors

	Subtype		
	β_1 (N = 3)	β_2 (N = 3)	β_3 (N = 3)
K_d (pM)	272 ± 1.9	433 ± 1.3	1638.9 ± 217.0
B_{max} (fmol/mg protein)	28.1 ± 1.5	29.8 ± 0.4	103.7 ± 7.6

Each datum represents the mean ± SEM. K_d: equilibrium dissociation constant of [^{125}I]cyanopindolol; B_{max}: maximal binding capacity of [^{125}I]cyanopindolol.

Table 2
Competition of [^{125}I]cyanopindolol binding with mexiletine and lidocaine in the membrane fraction prepared from Chinese hamster ovary cells expressing human β-adrenoceptors

	Subtype		
	β_1 (N = 3)	β_2 (N = 3)	β_3 (N = 3)
Mexiletine K_i (μM)	48.6 ± 10.8	62.7 ± 9.1	242.3 ± 79.7
Lidocaine K_i (μM)	807.7 ± 224.5	N.D.	N.D.

Each datum represents the mean ± SEM. K_i: equilibrium dissociation constant of mexiletine and lidocaine; N.D.: not detectable.

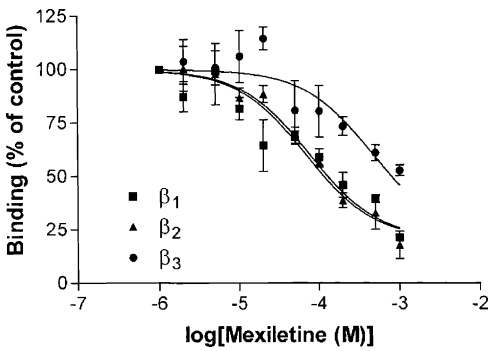

Fig. 1. Competitive radioligand binding of [^{125}I]cyanopindolol obtained with membrane fraction of Chinese hamster ovary cells expressing one of the β-adrenoceptor subtypes. Membrane fractions were incubated with a fixed concentration of [^{125}I]cyanopindolol (120 pM for β_1 and β_2; 1700 pM for β_3) in the presence of mexiletine. Each datum represents the mean ± SEM of three to four separate experiments.

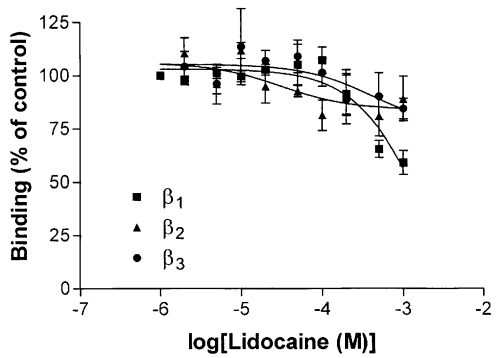

Fig. 2. Competitive radioligand binding of [^{125}I]cyanopindolol obtained with membrane fraction of Chinese hamster ovary cells expressing one of the β-adrenoceptor subtypes. Membrane fractions were incubated with a fixed concentration of [^{125}I]cyanopindolol (120 pM for β_1 and β_2; 1700 pM for β_3) in the presence of lidocaine. Each datum represents the mean ± SEM of three to four separate experiments.

3.2. Isoprenaline-stimulated cAMP accumulation

In order to determine functional properties of mexiletine and lidocaine, the isoprenaline-stimulated cAMP accumulation was measured in CHO-K1 cells expressing one of the β-adrenoceptor subtypes. As shown in Fig. 3, mexiletine reduces the isoprenaline-stimulated cAMP accumulation in CHO-K1 cells expressing either β_1- or β_2-adrenoceptors, but not β_3-adrenoceptors. In CHO-K1 cells expressing β_1-adrenoceptors, 5, 50, and 500 μM mexiletine has a similar inhibiting effect on the cAMP accumulation by 100 nM isoprenaline. In addition, the basal cAMP level was also significantly reduced by 500 μM mexiletine. In contrast to cells expressing β_1-adrenoceptors, mexiletine concentration-dependently inhibited the isoprenaline-stimulated cAMP accumulation in CHO-K1 cells expressing β_2-adrenoceptors. As shown in Fig. 4, lidocaine did not significantly alter the basal or the isoprenaline-stimulated cAMP accumulation in CHO-K1 cells expressing either β_1- or β_2-adrenoceptors. Both mexiletine and lidocaine did not reduce the basal and the isoprenaline-stimulated cAMP accumulation in CHO-K1 cells expressing β_3-adrenoceptors (Figs. 3C and 4C).

3.3. Forskolin-stimulated cAMP accumulation

Both mexiletine and lidocaine did not affect a forskolin-stimulated cAMP accumulation in CHO-K1 cells expressing one of the β-adrenoceptor subtypes (Figs. 5 and 6).

3.4. Effect of mexiletine on binding properties of [^{125}I]cyanopindolol to human β_1- and β_2-adrenoceptors

The results described above indicated that mexiletine acted as an antagonist for β_1- and β_2-adrenoceptors. To determine whether mexiletine competitively and reversibly displaces the binding of [^{125}I]cyanopindolol, the effect

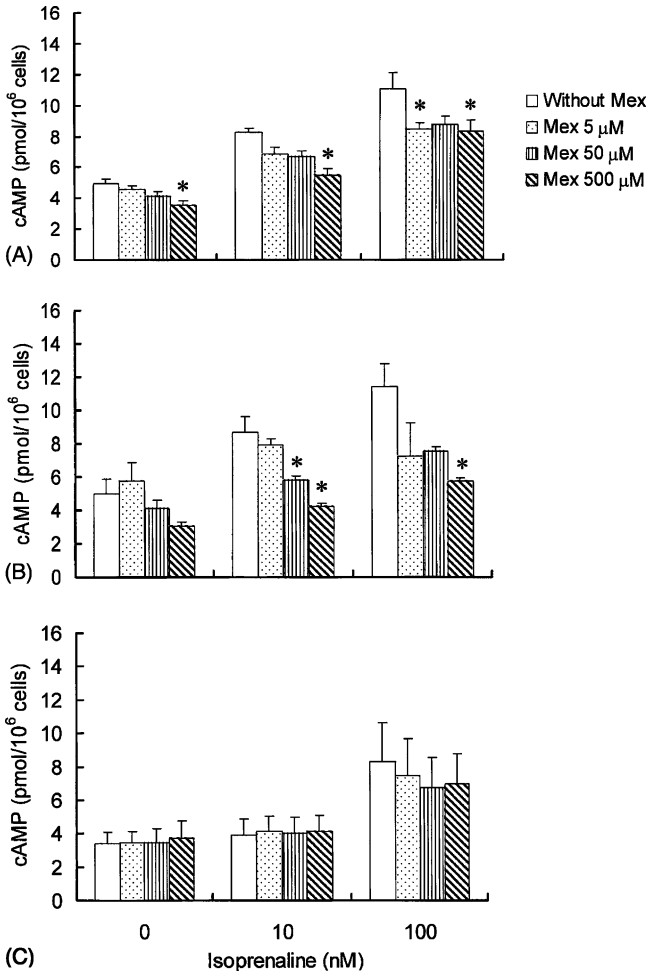

Fig. 3. Effect of mexiletine (Mex) on the isoprenaline-induced cAMP accumulation in Chinese hamster ovary cells expressing β_1-adrenoceptor (A), β_2-adrenoceptor (B), and β_3-adrenoceptor (C). Experiments were conducted in the presence of 0.5 mM 3-isobutyl-1-metylxanthine. Each column with a vertical bar represents the mean ± SEM of four to six separate experiments. $^*P < 0.05$ vs. corresponding without Mex values.

of mexiletine on binding properties of [^{125}I]cyanopindolol to human β_1- and β_2-adrenoceptors was investigated. Mexiletine decreased the B_{max} value but did not change the K_d value in human β_1- and β_2-adrenoceptors (Tables 3 and 4).

Table 3
Effect of mexiletine on binding properties of [^{125}I]cyanopindolol to the membrane fraction prepared from Chinese hamster ovary cells expressing human β_1-adrenoceptors

	Concentration of mexiletine			
	Control	5 μM	50 μM	500 μM
K_d (pM)	27.2 ± 1.9	55.7 ± 18.3	28.1 ± 7.0	21.7 ± 8.6
B_{max} (fmol/mg protein)	28.1 ± 1.5	14.8 ± 7.4	4.9 ± 0.9*	1.0 ± 0.4*

Each datum represents the mean ± SEM (N = 3). K_d: equilibrium dissociation constant of [^{125}I]cyanopindolol; B_{max}: maximal binding capacity of [^{125}I]cyanopindolol.
$^*P < 0.05$ vs. the value of the control group.

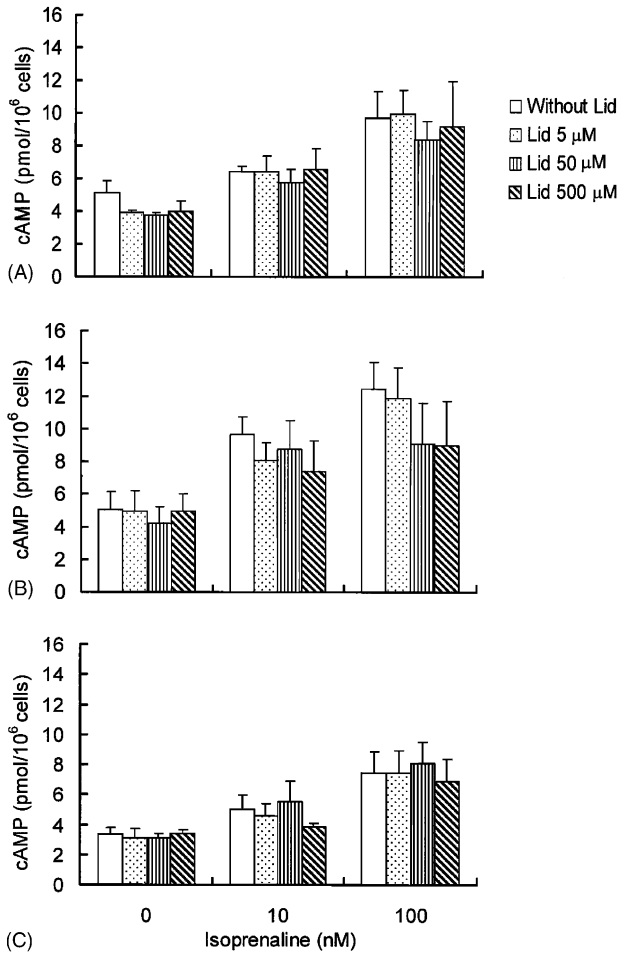

Fig. 4. Effect of lidocaine (Lid) on the isoprenaline-induced cAMP accumulation in Chinese hamster ovary cells expressing β_1-adrenoceptor (A), β_2-adrenoceptor (B), and β_3-adrenoceptor (C). Experiments were conducted in the presence of 0.5 mM 3-isobutyl-1-metylxanthine. Each column with a vertical bar represents the mean ± SEM of four to five separate experiments.

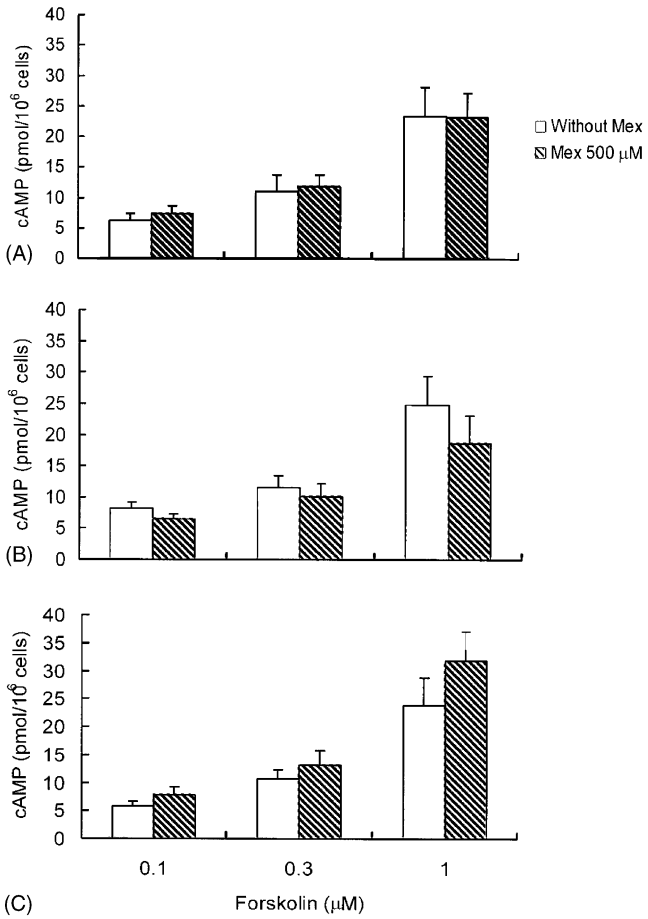

Fig. 5. Effect of mexiletine (Mex) on the forskolin-induced cAMP accumulation in Chinese hamster ovary cells expressing β_1-adrenoceptor (A), β_2-adrenoceptor (B), and β_3-adrenoceptor (C). Experiments were conducted in the presence of 0.5 mM 3-isobutyl-1-metylxanthine. Each column with a vertical bar represents the mean ± SEM of four to five separate experiments.

3.5. Effects of mexiletine on kinetic properties of [^{125}I]cyanopindolol bound to human β_1- and β_2-adrenoceptors

To test whether an allosteric mechanism are involved in the β_1- and β_2-antagonistic property of mexiletine, effects of mexiletine on the dissociation and association of [^{125}I]cyanopindolol were measured. The dissociation of [^{125}I]cyanopindolol from its binding site was monoexponential and only 500 μM mexiletine significantly accelerated the dissociation of radioligand from β_2-adrenoceptors, but not from β_1-adrenoceptors (Tables 5 and 6). Both 50 and 500 μM mexiletine significantly accelerated the association of radioligand to β_1- and β_2-adrenoceptors.

Table 4
Effect of mexiletine on binding properties of [^{125}I]cyanopindolol to the membrane fraction prepared from Chinese hamster ovary cells expressing human β_2-adrenoceptors

	Concentration of mexiletine			
	Control	5 μM	50 μM	500 μM
K_d (pM)	43.3 ± 1.3	41.4 ± 12.1	36.5 ± 12.4	36.1 ± 9.0
B_{max} (fmol/mg protein)	29.8 ± 0.4	15.8 ± 4.0*	6.6 ± 3.0*	5.0 ± 19*

Each datum represents the mean ± SEM (N = 3). K_d: equilibrium dissociation constant of [^{125}I]cyanopindolol; B_{max}: maximal binding capacity of [^{125}I]cyanopindolol.
*$P < 0.05$ vs. the value of the control group.

Table 5
Half-life ($t_{1/2}$) of dissociation and association of [^{125}I]cyanopindolol in the absence and presence of mexiletine in human β_1-adrenoceptors

	Concentration of mexiletine			
	Control	5 μM	50 μM	500 μM
$t_{1/2}$ dissociation (min)	0.38 ± 0.03	0.43 ± 0.04	0.36 ± 0.08	0.32 ± 0.03
$t_{1/2}$ association (min)	3.1 ± 0.2	3.1 ± 0.5	1.6 ± 0.1*	1.7 ± 0.1*

Each datum represents the mean ± SEM (N = 4).
*$P < 0.05$ vs. the value of the control group.

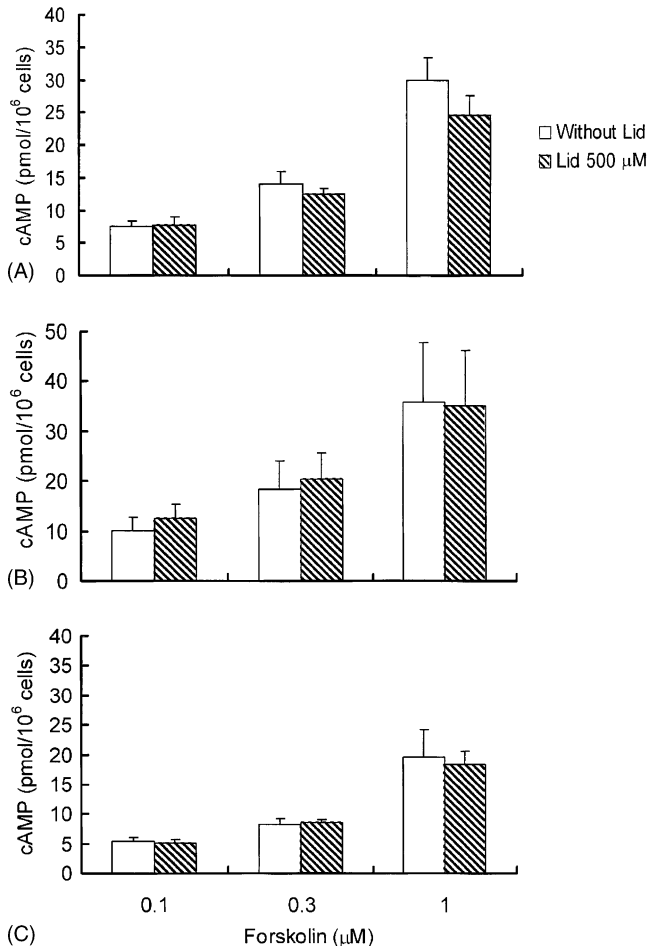

Fig. 6. Effect of lidocaine (Lid) on the forskolin-induced cAMP accumulation in Chinese hamster ovary cells expressing β_1-adrenoceptor (A), β_2-adrenoceptor (B), and β_3-adrenoceptor (C). Experiments were conducted in the presence of 0.5 mM 3-isobutyl-1-metylxanthine. Each column with a vertical bar represents the mean ± SEM of four to five separate experiments.

4. Discussion

The present study demonstrates for the first time that mexiletine can act as an antagonist for human β_1- and β_2-adrenoceptors. In a previous study, we have shown that mexiletine attenuates salbutamol-induced cAMP accumulation and relaxation of bovine tracheal smooth muscle through inhibition of specific binding of the agonist of β_2-adrenoceptors [3]. However, most tissues that predominantly express a certain β-adrenoceptor subtype usually contain some amount of other subtype(s). Thus, characterization of a drug with these tissues may be affected by small amounts of other adrenoceptor subtypes. The heterologous expression system using recombinant receptors is useful for evaluation of the binding properties of drugs without concerning populations of the expressed subtypes. In particular, this system enables us to characterize ligands for the receptors for which selective and high affinity radioligands are not available, such as β_3-adrenoceptor.

Table 6
Half-life ($t_{1/2}$) of dissociation and association of [^{125}I]cyanopindolol in the absence and presence of mexiletine in human β_2-adrenoceptors

	Concentration of mexiletine			
	Control	5 μM	50 μM	500 μM
$t_{1/2}$ dissociation (min)	0.54 ± 0.08	0.37 ± 0.09	0.35 ± 0.09	0.22 ± 0.05*
$t_{1/2}$ association (min)	3.2 ± 0.6	3.0 ± 0.6	1.5 ± 0.1*	1.3 ± 0.2*

Each datum represents the mean ± SEM (N = 4).
*$P < 0.05$ vs. the value of the control group.

The K_d values of [^{125}I]cyanopindolol in the present study were similar to that in the previous reports [7,11,12]. These results suggested that the β-adrenoceptor subtypes expressed in CHO-K1 cells retained normal pharmacological properties. The K_i value of mexiletine for β_1-adrenoceptors was almost the same as that for β_2-adrenoceptors, and five times smaller than that for β_3-adrenoceptors, indicating that the affinity of mexiletine for β_3-adrenoceptors is lower than that for β_1- and β_2-adrenoceptors. The K_i value of lidocaine, another class Ib antiarrhythmic drug, for β_1-adrenoceptors was 17 times lower than that of mexiletine. Moreover, lidocaine did not compete the binding of [^{125}I]cyanopindolol on β_2-adrenoceptors and β_3-adrenoceptors. Thus, mexiletine has a higher affinity to β-adrenoceptors than lidocaine. Although the reason why mexiletine acts as an antagonist for β-adrenoceptors is not clear, it is unlikely that the inhibitory effect of mexiletine on Na^+ channel contributes to its action as an antagonist of β-adrenoceptors.

In the present study, the bottom plateaus of the concentration–effect curves for the effects of mexiletine on radioligand binding did not reach the zero level. Therefore, there may not be competitive interplay between mexiletine and [^{125}I]cyanopindolol. However, if higher concentrations of mexiletine could have been tested, the curves might reach the zero level. (The maximum concentration of mexiletine we can dissolve is 500 μM.) To test whether mexiletine affects the K_d of radioligand binding while leaving its B_{max} unaffected as should be the case for a competitive interaction, the effects of mexiletine on the dissociation and association of [^{125}I]cyanopindolol were measured. Mexiletine concentration-dependently reduced the B_{max} value without affecting the K_d value in the CHO-K1 cells expressing β_1- and β_2-adrenoceptors. These results indicate that mexiletine is an irreversible antagonist for β_1- and β_2-adrenoceptors.

Isoprenaline stimulates β-adrenoceptors and then activates adenylate cyclase *via* a G_S protein-coupled mechanism, and forskolin stimulates the catalytic subunit of adenylate cyclase directly. In the present study, mexiletine reduced the isoprenaline-induced accumulation of cAMP in CHO-K1 cells expressing either β_1- or β_2-adrenoceptors, whereas it did not affect the forskolin-stimulated accumulation of cAMP in CHO-K1 cells expressing one of the

β-adrenoceptor subtypes. In addition, the basal cAMP level was also significantly reduced by 500 μM mexiletine in CHO-K1 cells expressing β_1-adrenoceptors. Moreover mexiletine has no effect on the basal content and the isoprenaline-induced accumulation of cAMP in CHO-K1 cells expressing β_3-adrenoceptors, suggesting mexiletine did not bind to β_3-adrenoceptors enough to reduce the basal content and the agonist-induced cAMP accumulation. Combined with the results of our radioligand binding experiments, mexiletine is likely to act as an antagonist of β_1- and β_2-adrenoceptors, and as an inverse agonist of β_1-adrenoceptors. The K_d value in the saturation binding experiments was about 30–40 pM. In the present study, 120 pM [^{125}I]cyanopindolol, four or five times higher concentration than K_d, was used for the competitive experiments in the CHO-K1 cells expressing β_1- and β_2-adrenoceptors. Therefore, higher concentration of mexiletine is needed for displacing [^{125}I]cyanopindolol than for reducing accumulation of cAMP.

Because mexiletine is a cationic amphiphilic agent, it tends to bind to lipophilic/hydrophilic interphases, such as phospholipids membranes [13]. In addition, there is a difference between β_1- and β_2-adrenoceptors in the inhibiting effect on the cAMP accumulation by isoprenaline. To test whether the allosteric mechanisms are involved in the β_1- and β_2-antagonistic property of mexiletine, effects of mexiletine on the dissociation and association of [^{125}I]cyanopindolol were measured. Mexiletine affected the dissociation of the radioligands for β_2-adrenoceptors, but not for β_1-adrenoceptors. Therefore, we concluded that it is possible that mexiletine affects β_2-adrenoceptors, but not β_1-adrenoceptors, by allosteric mechanisms. Allosteric mechanisms may explain the difference between β_1- and β_2-adrenoceptors in the inhibiting effect on the cAMP accumulation by isoprenaline.

Our previous study demonstrated that lidocaine augmented cAMP accumulation and relaxation induced by salbutamol and forskolin in bovine tracheal smooth muscle [4]. Studies on human lymphocytes showed that lidocaine inhibits the binding of ligands to β_2-adrenoceptors, and attenuates the production of the intracellular cAMP [5,6]. In the present study, lidocaine did not inhibit the binding of ligands to β-adrenoceptors enough to reduce the agonist-induced cAMP accumulation. The differences among observation in the present study and results of previous reports suggest that the effect of mexiletine and/or lidocaine on the signaling pathway mediated by β-adrenoceptors may depend on cell types and/or species.

Blood concentrations of mexiletine in human under treatment of arrhythmias range 0.5–2 μg/mL (i.e. 2.3–9.2 μM). Because the rate of mexiletine that is bound to plasma proteins is about 60%, the therapeutic concentration that will be available for an interaction with Na^+ channels and β-adrenoceptors is about 1.2–4.6 μM. Its K_i value for a cloned human Na^+ channel is 28.3 μM [14]. Thus, the concentration of mexiletine showing the inhibitory effect on β_1- and β_2-adrenoceptor-medicated responses is higher than its therapeutic concentration and K_i value for Na^+ channel. Therefore, the β-blocking effect of mexiletine is weaker than its Na^+ channel-blocking effect. Although β-antagonists have negative inotropic and chronotropic effects, they are reported to have a beneficial effect on early and chronic cardiac failure recently. Because mexiletine has a weak β-antagonistic effect as indicated above, cardiodepression induced by the drug is expected to be light. Thus, mexiletine may be a suitable drug for treatment of the patients suffering from both arrhythmia and early and chronic heart failure.

Even if the β-antagonistic effect of mexiletine is weak, overdosage of mexiletine, which is often a target for therapeutic drug monitoring, causes bradycardia and increase of the effect of β-antagonists. The β-antagonistic effect of mexiletine may be related to such side effect in overdosage of the drug. In addition, it may cause cardiodepression in such pathophysiological conditions as severe left ventricular dysfunction. It was shown that mexiletine caused cardiodepression in patients with severe left ventricular dysfunction [15,16] and a history of congestive heart failure [17]. Sodium channel-blocking effect as well as the β_1- and β_2-adrenoceptor-blocking effect of mexiletine may be able to explain these cardiodepression reported previously.

Previously, we reported that mexiletine attenuated β_2-agonist-induced relaxation on the bovine tracheal smooth muscle [3]. Moreover, mexiletine induced an increased dyspnea in patients with severe congestive heart failure [18]. Combined with the results in the present study, the therapy using mexiletine to the patient of asthma may have a risk.

Erythermalgia is a rare acrosyndrome characterized by reddening of the skin, local increase in heat and pain. This disease is frequently resistant to treatment and causes considerable suffering. Recently, Kuhnert *et al.* [19] reported favorable results that a combination of mexiletine and lidocaine had beneficial effect on the disease. More recently, Legroux-Crespel *et al.* [20] confirmed the beneficial effect of the combination, and demonstrated that mexiletine alone also had the beneficial effect. It is unknown whether the mechanism of the beneficial effect is peripheral, central, or even mixed. Its sodium channel-blocking effect as well as its β_1- and β_2-adrenoceptor-blocking effect may be involved in the underlying mechanism. Further studies are needed to examine a relationship between its beneficial effect on erythermalgia and its β_1- and β_2-adrenoceptor-blocking property.

In conclusion, we demonstrate for the first time that mexiletine blocks the binding of agonists to human β_1- and β_2-adrenoceptors, and thereby attenuates the agonist-induced cAMP accumulation. The action of mexiletine as an antagonist of human β_1- and β_2-adrenoceptors is independent of its antiarrhythmic property.

Acknowledgments

We would like to thank Dr. R.J. Lefcowitz (Duke University) for kindly providing the cDNA encoding β_1- and β_2-adrenoceptors. We are also grateful to Dr. Hitoshi Kurose and Dr. Taku Nagao (University of Tokyo) for kindly providing CHO-K1 cells expressing the β_1- and β_2-adrenoceptors and the cDNA encoding β_3-adrenoceptor. This study was supported by Kitasato University Research Grant for Young Researchers, and the Sasakawa Scientific Research Grant from The Japan Science Society (Tokyo, Japan).

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