Vasorelaxant effect of mexiletine in mesenteric resistance arteries of rats

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- 1 The vascular action of mexiletine, a class Ib antiarrhythmic agent, was investigated in the mesenteric resistance arteries of rats.
- 2 The second order branch of the mesenteric artery was cut into rings and changes in isometric tension were recorded.
- 3 Mexiletine (10^{-6} – 10^{-3} M) evoked concentration-dependent, endothelium-independent relaxations in arteries contracted with noradrenaline.
- 4 Mexiletine (10^{-4} M) did not affect the contraction induced by noradrenaline in Ca^{2+} -free solution, while the compound inhibited the contraction induced by CaCl_2 in noradrenaline-activated arteries.
- 5 The relaxation induced by mexiletine was less pronounced in arteries contracted with high KCl than in those contracted with noradrenaline.
- 6 Mexiletine induced identical relaxations in arteries contracted with noradrenaline in high KCl solution containing verapamil and in Krebs solution.
- 7 Thus, mexiletine induces relaxations by inhibiting transmembrane Ca^{2+} movement, but not Ca^{2+} release from the intracellular store site in mesenteric resistance arteries of rats. It is speculated that mexiletine possesses greater inhibitory effects against noradrenaline-activated, verapamil-insensitive (receptor-operated) Ca^{2+} channels than against verapamil-sensitive (voltage-dependent) channels.

Keywords: 5-Hydroxytryptamine; rat mesenteric artery; mexiletine; noradrenaline; relaxation

Introduction

Mexiletine is widely used for the treatment of life-threatening ventricular arrhythmias (Monk & Brogden, 1990; Nagasako *et al.*, 1992). Intravenous mexiletine is one of the drugs of choice for emergency treatment of the arrhythmias. The antiarrhythmic drug is classified as a class Ib agent (Vaughan Williams, 1984). Like other drugs in class-I category, mexiletine blocks sodium current in cardiac muscle cells, leading to an inhibition of the depolarization of the cells (Singh & Vaughan Williams, 1972; Shirayama *et al.*, 1991). The action of the drug might also cause inhibition against contractions in vascular smooth muscle. Indeed, lignocaine which bears some resemblance to mexiletine, evokes relaxation in vascular smooth muscle (Altura & Altura, 1974; Wang & Jackson, 1988; Perlmutter *et al.*, 1990). Stabilizing effects on cell membrane and antagonistic effects on movement of calcium ions have been proposed for the mechanisms of the vasodilatation by lignocaine (Altura & Altura, 1974). However, effects of mexiletine on vascular smooth muscle have not been intensively studied. It is speculated that the inhibitory effects of mexiletine on vascular smooth muscle might be greater than those of lignocaine since mexiletine has a considerable blocking action on Ca^{2+} channels besides its well known action on the Na^+ channel in ventricular cells (Ono *et al.*, 1986). Modulation of vascular tone by mexiletine would be of clinical importance if it occurs in small resistance arteries where peripheral blood flow is regulated. Indeed, a fall in blood pressure or a decrease in peripheral resistance after intravenous administration of mexiletine has been reported in man (Fritz *et al.*, 1983; Greenspan *et al.*, 1984).

The present study was designed to investigate the vascular effect of mexiletine and the interaction of the antiarrhythmic

drug with a neurotransmitter, noradrenaline, in mesenteric resistance arteries of rats.

Methods

Experimental animals and set-up

Male Wistar-Kyoto rats, 8–10 weeks of age, were obtained from Charles River Japan (Yokohama). The rat was stunned by a blow to the head and then exsanguinated. The entire mesenterium was removed and placed into cold modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118.6, KCl 4.8, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 25.1, disodium calcium edetate (EDTA) 0.026, and glucose 10.1; pH 7.4 (Krebs solution). The second order branch of the mesenteric artery was carefully dissected and cleaned of adhering adipose tissue and cut into rings (intraluminal diameter: 200 μm , width: 1.5 mm) under a dissection microscope. In some preparations, the endothelium was removed by rubbing the intimal surface with a thin glass rod (diameter: 200 μm). The presence or absence of the endothelium was confirmed later by the presence or absence of a relaxation to acetylcholine (10^{-9} – 10^{-6} M; Dohi *et al.*, 1990b). Unless otherwise stated in the text, the preparation used was without endothelium.

The ring preparation was suspended horizontally by means of two L-shaped stainless steel holders in the vessel lumen in a small chamber (5 ml) filled with Krebs solution (37°C) bubbled with a mixture of 95% O_2 and 5% CO_2 . One of the holders was fixed and the other was connected to a force-displacement transducer (UM-203, Kishimoto, Kyoto) for the measurement of isometric tension development. The ring was equilibrated under a resting tension of 200 mg for 60 min. This tension was found to be optimal for contraction of the preparation as assessed by repeated exposure to 60 mM KCl under various resting tensions (50–500 mg). After the equilibration period, the rings were maximally contracted by

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a 60 mM KCl solution two or three times at 45 min intervals until the responses were reproducible.

Protocol

Relaxation to mexiletine Concentration-response curves to mexiletine (10^{-6} – 10^{-3} M) were obtained in arteries with or without endothelium which were precontracted half-maximally with noradrenaline (NA), 5-hydroxytryptamine (5-HT), KCl, or calcium ionophore A23187 (A23187). In some experiments, the response to mexiletine was obtained in the presence of meclofenamic acid (10^{-5} M) to block the vascular production of prostaglandins (Vane, 1971; Diedrich *et al.*, 1990), glibenclamide (10^{-5} M) or tetraethylammonium (TEA; 10^{-2} M) to inhibit ATP- or Ca^{2+} -sensitive K^{+} channels, respectively (Sturgess *et al.*, 1985; Brayden & Nelson, 1992). The inhibitors were applied 30 min before the experiments began. In the experiments where effects of Na^{+} -free condition on the response to mexiletine were examined, the Krebs solution was replaced with a Na^{+} -free solution 20 min before the start of experiments.

Inhibitory effect of mexiletine against contractions Concentration-response curves to NA (3×10^{-8} – 3×10^{-4} M) or 5-HT (3×10^{-8} – 10^{-4} M) were constructed in arteries with or without endothelium. In arteries without endothelium, the first concentration-response curve was obtained in the absence of mexiletine, while the others were constructed in the presence of increasing concentrations of mexiletine (2.5×10^{-5} , 10^{-4} , 4×10^{-4} M). Arteries were exposed to mexiletine 20 min prior to the experiment. Results were plotted according to the method of Arunlakshana & Schild (1959) and the pA_2 value and slope of the regression for mexiletine versus NA or 5-HT were calculated. In time controls, the contraction induced by NA or 5-HT was reproducible throughout the experiment.

Caffeine-induced contractions were obtained by replacing the Krebs solution with a CaCl_2 -free solution containing 10^{-2} M caffeine. NA-induced contractions in Ca^{2+} -free solution were obtained by adding 10^{-5} M NA, 2 min after the Krebs solution was replaced with a Ca^{2+} -free solution. The contractions induced by caffeine or NA were reproducible at 20 or 45 min intervals, respectively. In another series of experiments, Ca^{2+} -induced contractions in NA-activated arteries were investigated. Arteries were contracted with caffeine by replacing the Krebs solution with the Ca^{2+} -free solution containing 10^{-2} M caffeine and washed with the Ca^{2+} -free solution for 25 min. Then, 10^{-5} M NA was added to the solution for 20 min and the solution was replaced with the CaCl_2 -free solution containing 10^{-5} M NA. After 2 min Ca^{2+} -induced contractions were obtained by addition of 2.5 mM CaCl_2 to the solution. These contractions were obtained in the presence or absence of 10^{-4} M mexiletine. Mexiletine was applied 20 min before contractions to caffeine or NA were obtained.

Drugs and solutions

The following drugs were used (unless otherwise stated from Sigma Chemical Co., St. Louis, Mo, U.S.A.): acetylcholine chloride, 4-bromo-calcium ionophore A23187 (A23187), caffeine, ethylene glycol-bis (β -aminoethyl ether) N,N,N',N' -tetraacetic acid (EGTA), glibenclamide (Yamanouchi Medical Pharmacy Co. Ltd., Tokyo, Japan), 5-hydroxytryptamine creatinine sulphate complex (5-HT), meclofenamic acid, mexiletine hydrochloride (Nippon Boehringer Ingelheim Co. Ltd., Kawanishi, Japan), (–)-noradrenaline bitartrate (NA), N -methyl-D-glucamine (NMG), tetraethylammonium chloride (TEA), verapamil (Eisai Co., Tokyo). The stock solutions of A23187 and glibenclamide were prepared in ethanol and dimethylsulphoxide (DMSO), respectively, while all other compounds were dissolved in distilled water or the Krebs solution. Drugs were applied cumulatively and concentra-

tions of drugs are expressed as final molar concentrations in the bath solution.

The CaCl_2 -free solution was made by omitting CaCl_2 and EDTA from the Krebs solution and the Ca^{2+} -free solution was made by adding 2 mM EGTA to the CaCl_2 -free solution. The high KCl solutions (40 and 60 mM KCl) were prepared by equimolar replacement of NaCl by KCl. A Na^{+} -free solution of the following composition (mM) was used: NMG 143.7, KCl 4.8, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.2 and glucose 10.1 (osmotic pressure of the solution was not different from that of the Krebs solution, pH was adjusted to 7.4 with HCl).

Calculations and statistics

Contractions were expressed as a percentage of the contraction developed by 60 mM KCl and relaxations were expressed as percentage inhibition of preceding contractions. Data are given as mean $\pm$ s.e.mean. The concentration of an agonist causing half-maximal contraction (EC_{50} value) or half-maximal inhibition of a preceding contraction (IC_{50} value) was calculated for each experiment and expressed as negative log molar (pD_2 value). The shift of concentration-response curves was expressed as concentration shift at pD_2 values. In each set of experiments, n equals the number of animals studied. Statistical analysis was by Student's paired and unpaired t tests and by analysis of variance followed by Scheffe's F test (Scheffe, 1959). Means were considered to be significantly different when the P value was less than 0.05.

Results

In quiescent arteries mexiletine (10^{-6} – 10^{-3} M) evoked no mechanical response ($n = 4$; data not shown).

Mexiletine (10^{-6} – 10^{-3} M) evoked concentration-dependent relaxations in the resistance arteries with endothelium, precontracted with NA (pD_2 value: 4.69 ± 0.05 , maximal response: $99.4 \pm 0.6\%$; Figure 1). Removal of the endothelium did not alter the response to mexiletine (pD_2 value: $4.61 \pm$

Figure 1 Concentration-response curves to mexiletine in mesenteric resistance arteries with (O) and without endothelium (●) contracted with noradrenaline obtained from 10–12 week old Wistar Kyoto rats. Note that the sensitivity and the maximal response to mexiletine are similar in arteries with and without endothelium. Each point represents mean $\pm$ s.e.mean from six experiments.

0.10, maximal response: $100 \pm 0\%$; NS). The relaxation to mexiletine was not affected by meclofenamic acid (10^{-5} M) ($n = 5$, data not shown). Mexiletine also reversed contractions induced by 5-HT in arteries without endothelium. The pD_2 value and the maximal response in arteries precontracted with 5-HT (pD_2 value: 4.68 ± 0.04 , maximal response: $99.3 \pm 0.6\%$, $n = 6$; NS) were not different from those obtained in arteries precontracted with NA.

In arteries without endothelium, NA (3×10^{-8} – 3×10^{-4} M) evoked concentration-dependent contractions (Figure 2a). Mexiletine (2.5×10^{-5} , 10^{-4} , 4×10^{-4} M) caused a significant shift to the right of the concentration-response curve to NA (Figure 2a, Table 1). Higher concentrations (10^{-4} , 4×10^{-4} M), but not a lower concentration of mexiletine (2.5×10^{-5} M) reduced the maximal response to NA (Table 1). In arteries

Figure 2 Concentration-response curves to noradrenaline (a) and 5-hydroxytryptamine (b) in the absence (○) and presence of increasing concentrations of mexiletine (2.5×10^{-5} M ●; 10^{-4} M △; 4×10^{-4} M □) in mesenteric resistance arteries without endothelium obtained from 10–12 week old Wistar Kyoto rats. Note that mexiletine causes a similar inhibition against the contraction induced by noradrenaline and 5-hydroxytryptamine. Each point represents mean from six experiments; s.e.mean shown by vertical bars.

with endothelium the contraction was also reduced by mexiletine (data not shown). The concentration shift by mexiletine (10^{-4} M) in arteries with and without endothelium was not different (5.0 ± 0.6 -fold and 4.5 ± 0.6 -fold, respectively; NS). Mexiletine also caused significant shifts of concentration-response curves to 5-HT (3×10^{-8} – 10^{-4} M) to the right in arteries without endothelium (Figure 2b, Table 1). The maximal response to 5-HT was reduced by higher concentrations of mexiletine (10^{-4} , 4×10^{-4} M). The concentration shift by mexiletine was not different in arteries contracted with NA (2.5×10^{-5} M: 1.4 ± 0.1 , 10^{-4} M: 4.5 ± 0.6 , 4×10^{-4} M: 33 ± 3 -fold) and 5-HT (2.5×10^{-5} M: 1.7 ± 0.2 , 10^{-4} M: 3.6 ± 0.1 , 4×10^{-4} M: 26 ± 2 -fold; NS). Schild analysis of the inhibitory effect of the three concentrations of mexiletine against NA and 5-HT revealed a slope (1.9 ± 0.3 , 1.4 ± 0.1 , respectively) that differed from 1.0 ($P < 0.01$) and pA_2 values of 4.27 ± 0.08 and 4.42 ± 0.08 , respectively. The pA_2 value and slope for mexiletine using NA as an agonist were not different from those obtained with 5-HT as an agonist.

Na^+ -free solutions shifted concentration-response curves to mexiletine to the right (Figure 3). The pD_2 value for mexiletine was smaller in the Na^+ -free solutions (4.31 ± 0.07) than in the Krebs solution (4.74 ± 0.05 ; $P < 0.001$), while the

Figure 3 Effect of Na^+ -free solution on concentration-response curves to mexiletine in mesenteric resistance arteries contracted with noradrenaline obtained from 10–12 week old Wistar Kyoto rats. The arteries were contracted half-maximally with noradrenaline in the Krebs solution (○) or the Na^+ -free solution (●) and relaxations to mexiletine were obtained. Note the small inhibition of the relaxation to mexiletine by the Na^+ -free solution. Each point represents mean $\pm$ s.e.mean from five experiments.

Table 1 Effect of mexiletine on pD_2 values and maximal responses for noradrenaline and 5-hydroxytryptamine

	Noradrenaline		5-Hydroxytryptamine	
	pD_2 value	max^a	pD_2 value	max^a
Control	6.21 ± 0.05	110 ± 5	6.85 ± 0.06	125 ± 5
With mexiletine:				
2.5×10^{-5} M	$6.05 \pm 0.06^*$	109 ± 5	$6.62 \pm 0.07^*$	124 ± 4
10^{-4} M	$5.57 \pm 0.05^*$	$100 \pm 3^*$	$6.29 \pm 0.05^*$	$114 \pm 4^*$
4×10^{-4} M	$4.69 \pm 0.03^*$	$69 \pm 2^*$	$5.45 \pm 0.03^*$	$65 \pm 4^*$

^aPercentage of 60 mM KCl-induced contraction

* $P < 0.05$ vs control.

maximal response was not different (99.3 ± 0.7 and $99.6 \pm 0.4\%$; NS).

Caffeine (10^{-2} M) induced transient contractions in the CaCl_2 -free solution (Figure 4a). The amplitude of the contraction was $42.5 \pm 6.3\%$ of the 60 mM KCl-induced contraction. Mexiletine did not alter the contraction to caffeine ($41.3 \pm 5.6\%$; NS; Figure 4c). NA (10^{-5} M) also evoked transient contractions in the Ca^{2+} -free solution ($58.4 \pm 4.9\%$; Figure 4b). Mexiletine did not change the contraction (64.1

Figure 4 (a) Contractile responses to caffeine in mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. The first response to 10 mM caffeine was assessed by replacing the Krebs solution with the CaCl_2 -free solution containing 10^{-2} M caffeine. Then, the arteries were washed out with the Krebs solution for 10 min, followed by treatment of the arteries with 10^{-4} M mexiletine in the solution for 20 min. The second contraction to caffeine was obtained in the presence of mexiletine. $[\text{CaCl}_2]_o$ represents concentrations of CaCl_2 in the solutions. (b) Contractile responses to noradrenaline in mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. The first response to 10^{-5} M noradrenaline (NA) was assessed in the Ca^{2+} -free solution after 2 min exposure of the arteries to the solution. After the contraction the arteries were washed out with the Krebs solution for 25 min and then treated with 10^{-4} M mexiletine in the solution for 20 min. The second contraction to noradrenaline was obtained in the Ca^{2+} -free solution in the presence of mexiletine after 2 min exposure to the solution. $[\text{Ca}^{2+}]_o$ represents concentrations of Ca^{2+} in the solutions. (c) Effect of mexiletine (open column: control, solid column: mexiletine 10^{-4} M) on the transient contraction induced by caffeine (10^{-2} M; in CaCl_2 -free solution) and noradrenaline (10^{-5} M; in Ca^{2+} -free solution) in mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. Note that mexiletine does not affect the contractions. Each column represents mean from five experiments; s.e.mean shown by vertical bars.

$\pm 6.2\%$; NS; Figure 4c). Replacement of the Ca^{2+} -free solution containing 10^{-5} M NA with Krebs solution evoked transient contractions, which were reduced by the pretreatment with mexiletine (Figure 4b).

Figure 5 (a) Contractile response to 2.5 mM CaCl_2 in mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. The arteries were contracted with caffeine by replacing the Krebs solution with the Ca^{2+} -free solution containing 10^{-2} M caffeine and washed out with the Ca^{2+} -free solution for 25 min. Then, 10^{-5} M noradrenaline (NA) was added to the solution for 20 min, and the solution was replaced with the CaCl_2 -free solution containing 10^{-5} M noradrenaline. After 2 min, the contraction to CaCl_2 was obtained by an application of 2.5 mM CaCl_2 . $[\text{CaCl}_2]_o$ represents concentrations of CaCl_2 in the solutions. (b) Effect of mexiletine on CaCl_2 -induced contraction in 10^{-5} M noradrenaline-activated mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. The same protocol as in (a) was carried out, but mexiletine was applied 20 min prior to the contraction to CaCl_2 . (c) Effect of mexiletine (open column: control, solid column: mexiletine 10^{-4} M) on the amplitude of 2.5 mM CaCl_2 -induced contraction in 10^{-5} M noradrenaline-activated mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. * $P < 0.01$. Each column represents mean with s.e.mean from five experiments.

After the application of caffeine, 10^{-5} M NA did not evoke contractions in the Ca^{2+} -free solution (Figure 5a) and the second application of caffeine at a 20 min interval was ineffective in the solution ($n = 5$, data not shown). Application of CaCl_2 (2.5 mM) to the CaCl_2 -free solution containing 10^{-5} M NA induced tonic contractions (Figure 5a), while application of caffeine (10^{-2} M) to the solution did not evoke contractions in time matched controls (data not shown; $n = 5$). The amplitude of the tonic contraction ($92.5 \pm 2.2\%$) was reduced by 10^{-4} M mexiletine ($66.1 \pm 6.8\%$; $P < 0.01$; Figure 5b,c).

Figure 6 Effect of contractile agents on concentration-response curves to mexiletine in mesenteric resistance arteries obtained from 10–12 week old Wistar Kyoto rats. The arteries were contracted half-maximally with noradrenaline (○), KCl (●), or calcium ionophore A23187 (□) and relaxations to mexiletine were obtained. Note that mexiletine induces less potent relaxations in arteries contracted with KCl than in those contracted with noradrenaline. Mexiletine does not relax the arteries precontracted with A23187. Each point represents mean $\pm$ s.e.mean from six experiments.

Figure 7 Relaxation to mexiletine in mesenteric resistance arteries precontracted with noradrenaline in the presence of verapamil obtained from 10–12 week old Wistar Kyoto rats. The arteries were contracted with 10^{-5} M noradrenaline (NA) and then 10^{-6} M verapamil was applied. After the relaxation to verapamil reached a steady state, mexiletine (Mex) was applied cumulatively. Numbers in the figure represent concentrations of mexiletine ($-\log M$). Typical tracing is shown in the figure from similar experiments ($n = 4$).

In arteries precontracted with 40 mM KCl (which caused half-maximal contractions) mexiletine caused less pronounced relaxations (pD_2 value: 3.62 ± 0.07 , maximal response: $88.2 \pm 2.9\%$) than in those precontracted with NA (pD_2 value: 4.60 ± 0.08 ; $P < 0.0005$, maximal response: $100 \pm 0\%$; $P < 0.05$; (Figure 6). Mexiletine did not induce relaxations in arteries precontracted with A23187 (Figure 6), while sodium nitroprusside (3×10^{-4} M) totally reversed the contraction ($n = 4$, data not shown). Verapamil (10^{-5} M) did not induce relaxations in arteries precontracted with A23187 ($n = 4$, data not shown).

In arteries precontracted with NA, mexiletine-induced relaxations (pD_2 value: 4.60 ± 0.08 , maximal response: $100 \pm 0\%$; $n = 5$) were not affected by 10^{-5} M glibenclamide (pD_2 value: 4.59 ± 0.07 , maximal response: $97.6 \pm 1.0\%$; $n = 5$;

Figure 8 (a) Relaxation to mexiletine in mesenteric resistance arteries precontracted with noradrenaline in the 40 mM KCl solution containing 10^{-6} M verapamil obtained from 10–12 week old Wistar Kyoto rats. The arteries were contracted with the 40 mM KCl solution and then 10^{-6} M verapamil was applied. After the tension reached the basal level, the arteries were contracted again half-maximally with noradrenaline (NA) and then relaxations to mexiletine (Mex) were obtained. Numbers in the figure represent concentration of mexiletine ($-\log M$). (b) Effect of KCl-induced depolarizations on mexiletine-induced relaxation in mesenteric resistance arteries contracted with noradrenaline obtained from 10–12 week old Wistar Kyoto rats. The arteries were contracted half-maximally with noradrenaline in the Krebs solution (○), with 40 mM KCl solution (●), or with noradrenaline in 40 mM KCl solution containing 10^{-6} M verapamil (□) and concentration-response curves to mexiletine were obtained. Note the similar relaxations to mexiletine in arteries contracted with noradrenaline in the Krebs solution and in the 40 mM KCl solution containing 10^{-6} M verapamil. Each point represents mean $\pm$ s.e.mean from six experiments.

NS) or 10^{-2} M TEA (pD_2 value: 4.58 ± 0.06 , maximal response: 98.7 ± 0.9 ; $n = 5$; NS).

Contractions induced by 10^{-5} M NA were partially reversed by 10^{-6} M verapamil (Figure 7). The residual contraction after the application of the Ca^{2+} antagonist (verapamil-insensitive part; approximately a half of the maximal contraction to NA) was reversed by mexiletine (Figure 7). The pD_2 value and the maximal response to mexiletine in the condition were not different from those obtained in Figure 1 (data not shown).

Contractions induced by 40 mM KCl were totally reversed by 10^{-6} M verapamil (Figure 8a). After the tension reached the basal level by verapamil, the artery was again contracted half-maximally with NA and then the relaxation to mexiletine was investigated. In these conditions, mexiletine evoked relaxations with similar pD_2 value (4.56 ± 0.09) and maximal response ($100 \pm 0\%$) to those obtained in arteries contracted with NA in the Krebs solution (pD_2 value: 4.75 ± 0.04 , maximal response: $99.6 \pm 0.4\%$; NS), while the responses to 3×10^{-5} and 10^{-4} M mexiletine were different in the two groups (3×10^{-5} M: 53.9 ± 7.1 and $80.6 \pm 7.3\%$; $P < 0.05$, 10^{-4} M: 82.9 ± 3.1 and $97.3 \pm 1.9\%$; $P < 0.005$; Figure 8b). The relaxation was more pronounced in arteries precontracted with NA in the 40 mM KCl solution containing verapamil than in those precontracted with 40 mM KCl (pD_2 value: 3.47 ± 0.10 ; $P < 0.0005$, maximal response: $79.7 \pm 5.5\%$; $P < 0.01$; Figure 8b).

Discussion

Mexiletine induced concentration-dependent relaxations in mesenteric resistance arteries precontracted with NA. Removal of the endothelium did not alter the relaxation, indicating that the relaxation is not endothelium-dependent. Mexiletine also shifted the concentration-response curves for NA and 5-HT to the right. Since the maximal responses to the agonists were reduced by higher concentrations of mexiletine and the slopes of the Schild plot were significantly different from 1.0, mexiletine does not act as a competitive α -adrenoceptor or 5-HT receptor antagonist. Furthermore, mexiletine shifted concentration-response curves to NA and 5-HT with identical pA_2 values and slopes. This suggests that NA and 5-HT activate a common pathway in pharmacomechanical (receptor-contraction) coupling and that mexiletine acts on the common pathway which is distal to the receptors. Indeed, mexiletine caused similar relaxations in arteries precontracted with NA and 5-HT. Involvement of prostaglandins in the relaxation to mexiletine can also be excluded since the cyclooxygenase inhibitor, meclofenamic acid, did not affect the relaxation.

There are only few reports investigating vascular effects of mexiletine: mexiletine induces small contractions in human saphenous vein, but not in arteries (Chiavarelli *et al.*, 1990), and does not affect NA-induced contraction in guinea-pig aorta (Kitagawa *et al.*, 1985). The discrepancy between the present observation and previous work in which large vessels were used can be explained, at least in part, by the difference in species or anatomical origin of the preparations. In contrast to mexiletine, there are many reports investigating vascular effects of lignocaine which possesses a chemical structure and pharmacological effects similar to those of mexiletine. The local anaesthetic causes relaxations (Altura & Altura, 1974; Wang & Jackson, 1988; Perlmutter, 1990) and, in some preparations, contractions (mainly in venous smooth muscles: Altura & Altura, 1974; Johns *et al.*, 1985; Monuszko *et al.*, 1989). Although inhibition of myosin light-chain kinase (Nosaka *et al.*, 1989) and phospholipase A_2 (Kunze *et al.*, 1976; Willman & Hendrickson, 1978), and antagonistic effects on movement of calcium ions (Josephson & Sperelakis, 1976) in smooth muscle by lignocaine have been reported, local anaesthetics are generally believed to induce

nonspecific inhibition of vascular tone (Altura & Altura, 1974).

Caffeine induced transient contractions in $CaCl_2$ -free solution, as in previous reports, by releasing Ca^{2+} from intracellular Ca^{2+} store site (sarcoplasmic reticulum; Endo, 1977; Dohi *et al.*, 1990a). Mexiletine did not change the contraction, indicating that mexiletine does not affect, at least at the concentration used, the release of Ca^{2+} . NA also evoked transient contractions in Ca^{2+} -free solution, confirming previous observations (Itoh *et al.*, 1983; Dohi *et al.*, 1990a) and suggesting that the contraction to NA in Ca^{2+} -free solution is independent of extracellular Ca^{2+} . The fact that mexiletine did not alter the contraction indicates that mexiletine does not inhibit the release of Ca^{2+} from the NA-sensitive Ca^{2+} store. In contrast, $CaCl_2$ -induced contractions in NA-activated vessels were inhibited by mexiletine. The contraction was evoked by extracellular Ca^{2+} since the NA-sensitive intracellular Ca^{2+} store had been depleted (NA did not evoke contraction before the addition of $CaCl_2$ and application of caffeine instead of $CaCl_2$ did not evoke contraction). Thus, mexiletine inhibits NA-induced contraction by inhibiting the component of the contraction which depends on extracellular, but not intracellular Ca^{2+} .

A23187 produces a contraction which is dependent on the external Ca^{2+} in smooth muscles (Ishida & Shibata, 1980) by increasing the permeability of the membrane to Ca^{2+} (Swamy *et al.*, 1975). However, the contraction induced by A23187 was insensitive to verapamil and mexiletine did not induce relaxations in arteries contracted with A23187, indicating that mexiletine does not nonspecifically inhibit contractions which depend on extracellular Ca^{2+} . The compound might inhibit Ca^{2+} movement through Ca^{2+} channels. Indeed, in single ventricular cells of guinea-pig, mexiletine is effective in blocking Ca^{2+} channels (Ono *et al.*, 1986).

High K^+ depolarizes the membrane of vascular smooth muscle cells. In the present study, the contraction induced by 40 mM KCl was completely reversed by verapamil, indicating that the contraction depends on Ca^{2+} influx through verapamil-sensitive (voltage-dependent) Ca^{2+} channels. Mexiletine evoked less pronounced relaxations in arteries precontracted with KCl than in arteries precontracted with NA. This raises the following possibilities: (1) mexiletine causes the relaxation by hyperpolarizing the membrane (hyperpolarization might be prevented by KCl-induced depolarization), (2) mexiletine causes relaxations by opening K^+ channels (excess $[K^+]_o$ might prevent K^+ efflux through K^+ channels), and (3) inhibitory effects of mexiletine are more potent against NA- (or 5-HT-) sensitive Ca^{2+} channels than against verapamil-sensitive Ca^{2+} channels.

During the contraction to NA in arteries activated with high KCl in the presence of verapamil, the membrane potential of the mesenteric artery might be fixed by KCl-induced depolarization (Nagao & Vanhoutte, 1991). Even in this condition, mexiletine induced potent relaxations, indicating that KCl-induced depolarization itself has only minor effects on the relaxation to mexiletine. This suggests that a large part of the relaxation to mexiletine might not be due to hyperpolarization of the membrane caused by the drug. Although mexiletine inhibits depolarization of the membrane in cardiac muscle cells (Singh & Vaughan Williams, 1972; Shirayama *et al.*, 1991), the inhibitory effect against depolarization does not play a major role in the relaxation of the vascular smooth muscle. Mexiletine-induced relaxations in arteries contracted with NA in the high KCl solution containing verapamil were much more potent than in arteries contracted with high KCl. This finding further reinforces the concept that a large part of the inhibitory effect of mexiletine is not related to the hyperpolarizing effect of the drug. Mexiletine must have a greater inhibitory effect against NA- (or 5-HT-) activated, verapamil-insensitive Ca^{2+} channels than against verapamil-sensitive channels since the NA-induced contraction in the high KCl solution containing verapamil depends on Ca^{2+} influx through NA-activated, but not vera-

pamil-sensitive Ca^{2+} channels. Moreover, the fact that the pA_2 values (and slopes) for mexiletine using NA and 5-HT as agonists were identical suggests that 5-HT may act on NA-sensitive Ca^{2+} channels which might be the most important target of the antiarrhythmic drug in the vasorelaxant action. The failure of TEA and glibenclamide to inhibit the relaxation to mexiletine indicates that an activation of K^+ channels (at least those of TEA- and glibenclamide-sensitive) which may evoke relaxations by hyperpolarizing the membrane of the vascular smooth muscle is not involved in the relaxation.

In Na^+ -free solution, where effects of Na^+ -channel blockade may disappear, the relaxation to mexiletine was slightly inhibited. This suggests that mexiletine-induced relaxation might be related, at least in part, to Na^+ channel blockade which has hyperpolarizing effects on the membrane. Indeed, KCl-induced depolarization (in the presence of verapamil), which may reduce the hyperpolarizing effect of Na^+ channel blockade by fixing the membrane potential, slightly reduced the relaxation (see Figure 8). However, the Na^+ channel blockade contributes to only a small part of the relaxation induced by mexiletine.

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Although mexiletine induces a fall in arterial pressure in dogs (Rossi *et al.*, 1984) and a decrease in total peripheral resistance and arterial pressure in rabbits (Beltrame *et al.*, 1984), it is not clear whether the vascular effect of mexiletine is clinically important especially since the effective concentration of mexiletine in the present study is slightly higher than the therapeutic concentration in man (2×10^{-6} – 10^{-5} M; Vozeh *et al.*, 1982; Woosley *et al.*, 1984; Monk & Brogden, 1990). However, the possibility that the antiarrhythmic drug causes vasodilatation in man cannot be excluded. Indeed, reduction in blood pressure or total peripheral resistance has been reported in man (Fritz *et al.*, 1983; Greenspan *et al.*, 1984).

In conclusion, mexiletine induces potent relaxations in mesenteric resistance arteries contracted with NA. The mechanisms underlying the relaxation to mexiletine are related to the inhibitory effects of the drug against Ca^{2+} influx, but not release caused by NA. It is speculated that mexiletine possesses greater inhibitory effects against NA-activated, verapamil-insensitive (receptor-operated) Ca^{2+} channels than against verapamil-sensitive (voltage-dependent) Ca^{2+} channels.

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