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Direct Inhibitory Effect of Chlorpromazine on Smooth Muscle of the Porcine Pulmonary Artery

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Background: **Chlorpromazine has been widely used by anesthesiologists to take advantage of its anesthesia-potentiating and vasorelaxing actions. However, the mechanisms of vasorelaxation induced by chlorpromazine are still not fully understood.**

Methods: **Using front-surface fluorometry of fura-2 and porcine pulmonary arterial strips, we investigated the effects of chlorpromazine on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and force of vascular smooth muscle. The affinities of chlorpromazine and other neuroleptics to vascular α_1-adrenergic receptors were then determined by a radio-ligand binding study.**

Results: **Chlorpromazine (as much as 1 μM) inhibited both the elevation of $[Ca^{2+}]_i$ and force in pulmonary arterial smooth muscle induced by 80 mM K^+-depolarization and 1 μM norepinephrine in a concentration-dependent manner. The extent of inhibition by chlorpromazine in norepinephrine-induced contraction was much greater than that in 80 mM K^+-induced contraction. In contrast, as much as 1 μM chlorpromazine had no effect on the increases in $[Ca^{2+}]_i$ or force induced by U46619, a thromboxane A_2 analogue. Chlorpromazine also had no effect on the intracellular Ca^{2+} release induced by U46619. In a radio-ligand displacement study, chlorpromazine, haloperidol, phentolamine, trifluoperazine, and imipramine inhibited the specific binding of [^{3}H] prazosin to the porcine aortic membranes, in this order of potency.**

Conclusions: **Chlorpromazine induces vasorelaxation through an α-adrenergic blocking action as well as a calcium antagonistic action; the former action may, therefore, play a major role in chlorpromazine-induced vasorelaxation. (Key words: Anesthetics, general: neuroleptics; chlorpromazine. Receptors, adrenergic: α-adrenergic receptor. Ions: calcium. Muscle, smooth: vascular. Assay, binding, radioligand: [^{3}H]-prazosin.)**

AN important action of neuroleptic drugs, including chlorpromazine, is their ability to relax vascular smooth muscle. The vasorelaxing actions of these drugs have been attributed to the effects on the central nervous system and peripheral blood vessels.[1] For the peripheral effect, it has been generally accepted that these drugs have α-adrenergic blocking property.[1-3] However, this peripheral α-adrenergic blocking property is based on the observation that many neuroleptics inhibit the radioligand binding to α-adrenergic receptors in the brain membrane preparation.[4-8] Therefore, the evidence that neuroleptics inhibit the radioligand binding to α-adrenergic receptors of the vascular smooth muscle has yet to be reported. In addition to the α-adrenergic blocking property, it was reported that chlorpromazine has a calcium antagonistic property in vascular smooth muscle.[9,10] Therefore, it appears that chlorpromazine has at least two mechanisms as a vasorelaxant; however, no direct evidence indicating the relative importance of these two mechanisms for vasorelaxation has yet to be described.

In the current study, we investigated the effects of chlorpromazine on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), using front surface fluorometry with fura-2,[11,12] and force development in porcine pulmonary

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artery. We also examined the neuroleptic-induced inhibition of [^{3}H]prazosin binding to vascular α_1-adrenergic receptors, to determine the relative importance of α-adrenergic receptor blockade and Ca^{2+} antagonist properties in the mechanism of vasorelaxation. Based on our findings, we provide direct evidence for the neuroleptic-induced inhibition of [^{3}H] prazosin binding to vascular α_1-adrenergic receptors.

Materials and Methods

Tissue Preparation for Measurement of Force Development and Front-Surface Fluorometry

The lungs of adult pigs were obtained from a local slaughterhouse immediately after the pigs had been killed and were transported to our laboratory in preaerated ice-cold physiologic saline solution (PSS). The intrapulmonary arteries were dissected from lungs and cut longitudinally. The endothelium was removed by rubbing the inner surface with a cotton swab, and the adventitia was trimmed away using a microscope. The muscle sheets were transversely cut into strips measuring 5 mm in length, 1 mm in width, and approximately 0.1 mm in thickness. All tissue preparations were performed in oxygenated (95% O_2 and 5% CO_2) PSS.

The strips were then loaded with fura-2, in the form of acetoxymethyl ester (fura-2/AM). The strips were incubated in Dulbecco-modified Eagle's medium that contained 25 μM fura-2/AM dissolved in dimethyl sulphoxide and 5% fetal bovine serum[11,12] for 4 h at 37°C. After loading with fura-2, the strips were rinsed with normal PSS to remove the dye in the extracellular space and to equilibrate them for at least 60 min at 37°C before starting the measurements.

Simultaneous Measurements of Force Development and $[Ca^{2+}]_i$

The fura-2-loaded strips were mounted vertically in a quartz organ bath and connected to a strain gauge (TB-612T, Nihon Koden, Japan). During the 60-min fura-2 equilibration period, the strips were stimulated with 80 mM K^+-depolarization every 15–20 min, and the resting tension was increased in a stepwise manner to obtain the maximal force development. The appropriate resting tension level obtained by this procedure was approximately 300–400 mg. The responsiveness of each strip to 80 mM K^+ was recorded before starting the experimental protocol. The developed force was expressed in percentage, assigning the values in normal (5.9 mM K^+) PSS and steady state of 80 mM K^+-PSS to be 0 and 100%, respectively.

Changes in the fluorescence intensity of the fura-2-Ca^{2+} complex were monitored by using a front-surface fluorometer specifically designed by us for fura-2 fluorometry (model CAM-OF) with the collaboration of Japan Spectroscopic (Tokyo, Japan).[11,12] In brief, dual wavelength excitation light (340 and 380 nm) was obtained from a spectroscope from a xenon light source. By using a chopper wheel, the excitation light was alternately (400 Hz) guided through quartz optic fibers arranged in a concentric inner circle (diameter = 3 mm) and which directly illuminated the entire vascular strip. The surface fluorescence of the strip was collected by glass optic fibers arranged in an outer circle (diameter = 7 mm) and introduced through a 500 ± 10 nm band-pass filter into a photomultiplier. The ratio (F_{340}/F_{380}) of the fluorescence intensities at 340 nm excitation (F_{340}) to those at 380 nm excitation (F_{380}) was monitored and expressed as a percentage, assigning the values at rest in normal (5.9 mM K^+) and in depolarization with 80 mM K^+-PSS to be 0% and 100%, respectively. The percent values of the fluorescence ratios were used for the statistical analysis of $[Ca^{2+}]_i$. As for the reference, the absolute value of $[Ca^{2+}]_i$ was determined as follows. The minimum and maximum fluorescence ratios were determined by the addition of 25 μM ionomycin to Ca^{2+}-free PSS that contained 2 mM ethyleneglycol-*bis* (β-aminoethylether) N,N,N′,N′-tetraacetic acid (EGTA), followed by replacement with normal PSS, respectively. The absolute value of $[Ca^{2+}]_i$ was then calculated in a separate measurement using the equation of Grynkiewicz *et al.*[13] The calculated $[Ca^{2+}]_i$ in normal PSS (0%) and steady state of 80 m MK^+-PSS (100%) were 86.6 ± 9.8 and 508 ± 67.3 nM (n = 10) at 37°C, respectively.

Experimental Protocols

To examine the effects of chlorpromazine on the high K^+-, norepinephrine-, or U46619 (a thromboxane A_2 analogue)-induced changes in $[Ca^{2+}]_i$ and force development, various concentrations of chlorpromazine were applied 10 min before and during the high K^+-, norepinephrine-, or U46619-induced contraction. To examine the effects of chlorpromazine on the intracellular Ca^{2+} release, the strips were treated in Ca^{2+}-free solution that contained 2 mM EGTA and stimulated by U46619 in the presence or absence of chlorpromazine. In the current study, we used chlorpromazine at the concentration ranging from 10 nM to 1 μM, which

could be regarded as the possible therapeutic concentration.[14,15]

Radioligand Binding Assay

For each preparation of aortic membranes, 5–6 porcine thoracic aortas were obtained from a local slaughterhouse immediately after the pigs had been killed and were transported to our laboratory in ice-cold buffer composed of 0.25 M sucrose, 10 mM morpholinopropanesulfonic acid, and 0.05% bovine serum albumin (pH 7.4). The aortas were opened longitudinally, the first intima layer scraped off, and the media layer stripped from the adventitia. The microsomal fraction was prepared as described previously.[16] The trimmed media layers were finely minced and homogenized in the buffer with a Polytron PT 10 homogenizer. The homogenate was centrifuged at 900 × g for 10 min, the supernatant was centrifuged at 9,000 × g for 20 min, and then again at 108,000 × g for 45 min. The resultant pellet was resuspended in 0.25 M sucrose solution that contained 10 mM morpholinopropanesulfonic acid (pH 7.4), recentrifuged at 9,000 × g for 10 min, and the supernatant centrifuged at 178,000 × g for 45 min. The resultant pellet (microsomal fraction) was suspended in 50 mM Tris buffer (pH 7.4) for the binding study. The protein was assayed according to the method of Markwell *et al.*,[17] using bovine serum albumin as a standard.

The dissociation constant (K*d*) and the maximum binding (*B* max) values determined by the Scatchard analysis in microsomal fraction were 0.069 nM and 273 fmol/mg protein, respectively, as reported previously.[16] In the displacement study, 100 μg protein of the aortic microsome and 0.1 nM [^{3}H] prazosin were incubated with various concentrations of the indicated drugs at 25°C for 30 min in a total volume of 1 ml that contained 50 mM Tris and 5 mM $MgCl_2$ (pH 7.4), with or without 10 μM phentolamine, to determine the nonspecific or total binding, respectively. Binding was terminated by adding 5 ml ice-cold buffer and filtered onto Whatman GF/C glass fiber filters, with 3 × 5 ml washes with ice-cold buffer. Specific binding was defined as the total binding minus nonspecific binding. All binding assays were carried out either in duplicate or triplicate.

Data Analysis

All data for the simultaneous measurements of $[Ca^{2+}]_i$ and force were collected with a computerized data acquisition system (MacLab; Analog Digital instruments, Castle Hill, Australia; and Macintosh, Apple Computer, Cupertino, CA). The data for the representative traces shown in this report were directly printed from a computer to a laser printer (LaserWriter II NTX-J, Apple Computer). The measured values were expressed as the means ± SE (n = number of the experiments). For each experiment, a strip from a different animal (3–8) was used. Unpaired Student's *t* test was used to determine the statistical significance. Analysis of variance (ANOVA) was used to determine the concentration-dependency of effects of chlorpromazine. *P* values less than 0.05 were considered significant.

All data for binding study were analyzed as follows. The inhibition constant (K*i*) was determined from the formula of Cheng and Prusoff,[18] $Ki = IC_{50}/(1 + A/Kd)$, where A = radioligand concentration, K*d* = dissociation constant, and IC_{50} = concentration of competitive ligand that inhibits radioligand binding by 50%. The IC_{50} values were determined from the competition curves by the 4-parameter logistic equation of DeLean *et al.*[19] The data are expressed as the means ± SE. The group mean values were compared using the two-tailed Student's *t* test.

Solutions and Drugs

Normal PSS was of the following composition (in mM): NaCl 123, KCl 4.7, $NaHCO_3$ 15.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.25, and D-glucose 11.5. High K^+ PSS was identical to normal PSS, except for an equimolar substitution of KCl for NaCl. Physiologic saline solution was bubbled with 95% O_2 and 5% CO_2, with a resulting pH of 7.4 at 37°C. Fura-2 AM, U46619, and EGTA were purchased from Dojindo (Kumamoto, Japan). Chlorpromazine was from Wako (Osaka, Japan). Phentolamine was a gift from Ciba-Geigy (Osaka, Japan). [^{3}H] prazosin (specific activity = 80.9 C*i*/mmol) was purchased from New England Nuclear (Boston, MA). All other agents were purchased from Sigma Chemical (St. Louis, MO).

Results

Effects of Chlorpromazine on the Increases in $[Ca^{2+}]_i$ and Force Induced by 80 mM K^+-Depolarization

Figure 1A shows the representative recordings of the changes in the intensity of 500 nm fluorescence at 340 (F_{340}) and 380 nm (F_{380}) excitation, the ratio (F_{340}/F_{380}) and force induced by 80 mM K^+-PSS of the porcine

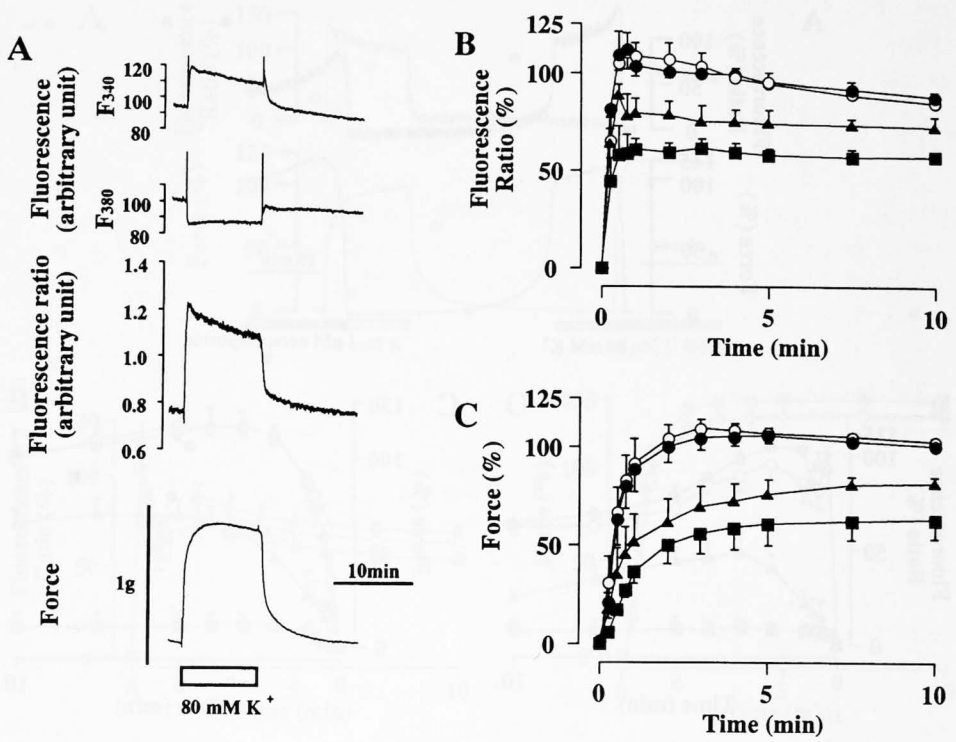

Fig. 1. Effects of chlorpromazine on the increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and force induced by 80 mM K^+-depolarization. (*A*) Representative recordings showing changes in the fluorescence and force development induced by 80 mM K^+-depolarization. The first and second traces from the top show changes in the 500-nm fluorescence intensities obtained at 340 nm (F_{340}) and 380 nm (F_{380}) excitations, respectively. The third trace shows changes in the fluorescence ratio of F_{340} to F_{380}. The lowest trace shows the force development. (*B and C*) Effects of various concentrations of chlorpromazine on changes in $[Ca^{2+}]_i$ (*B*) and force development (*C*) induced by 80 mM K^+-depolarization. The strips were pretreated with 0 M (○; control), 10 nM (●), 100 nM (▲), and 1 μM (■) chlorpromazine. Chlorpromazine was applied for 10 min before and was present during 80 mM K^+-depolarization. The abscissa scales in (*B*) and (*C*) show the time (in min) after the application of 80 mM K^+–physiologic saline solution. The mean values with SE bars are shown (n = 3–5). The number of experiments refers to the number of strips from each different animal.

pulmonary arterial strips. Depolarization with 80 mM K^+-PSS induced a rapid increase in F_{340} and a decrease in F_{380} as a mirror image, which ensures the specificity of fura-2 signal. After a rapid increase, the ratio ($[Ca^{2+}]_i$) reached a peak, and then declined to reach a plateau level. The force also rapidly elevated to reach a plateau level. In all the fura-2 measurements, we monitored F_{340} and F_{380} routinely and confirmed that F_{340} and F_{380} showed a mirror image. Except in Figure 1A, only the fluorescence ratio was illustrated to indicate $[Ca^{2+}]_i$. When the strips were pretreated with various concentrations of chlorpromazine during the resting state in normal PSS, no significant changes in $[Ca^{2+}]_i$ and force were observed (data not shown). Figures 1B and 1C show the effects of various concentrations of chlorpromazine on the increases in $[Ca^{2+}]_i$ and force induced by 80 mM K^+-PSS. Chlorpromazine inhibited the elevation of $[Ca^{2+}]_i$ and force induced by 80 mM K^+-depolarization in a concentration-dependent manner ($P < 0.05$, by two-way ANOVA). Chlorpromazine at the concentration of 1 μM inhibited 80 mM K^+-induced increases in $[Ca^{2+}]_i$ and force to 60.6 ± 4.6% and 66.5 ± 6.2% (n = 5), respectively, while assigning the value during depolarization with 80 mM K^+ PSS to be 100%. Therefore, chlorpromazine at the highest concentration examined (1 μM) could only partially reduce the elevated $[Ca^{2+}]_i$ and force induced by 80 mM K^+-depolarization. After washing out chlorpromazine with normal PSS, 80 mM K^+-PSS induced the same extent of response in $[Ca^{2+}]_i$ and force as the control value (data not shown), which indicated that the effect of chlorpromazine is reversible.

Effects of Chlorpromazine on the Increases in $[Ca^{2+}]_i$ and Force Induced by Norepinephrine in Normal Physiologic Saline Solution

When 1 μM norepinephrine was applied in normal PSS, $[Ca^{2+}]_i$ rapidly increased to form a peak (first phase) and then $[Ca^{2+}]_i$ gradually declined, but remained at a level higher than the prestimulation level (second phase). The force also developed rapidly to reach a peak and then declined gradually (fig. 2A). The $[Ca^{2+}]_i$ and force observed at maximum level were 106.5 ± 6.8% and 116.3 ± 9.6%, respectively, of those induced by 80 mM K^+-depolarization. Therefore, the maximum levels of $[Ca^{2+}]_i$ and force observed in the contraction induced by norepinephrine were either similar or slightly higher than those induced by 80 mM K^+-depolarization. The $[Ca^{2+}]_i$ and force observed at the second phase (10 min after the application) were 63.1 ± 5.4% and 97.9 ± 15.4%, respectively (n = 3). Figures 2B and 2C show the effects of various concentrations of chlorpromazine

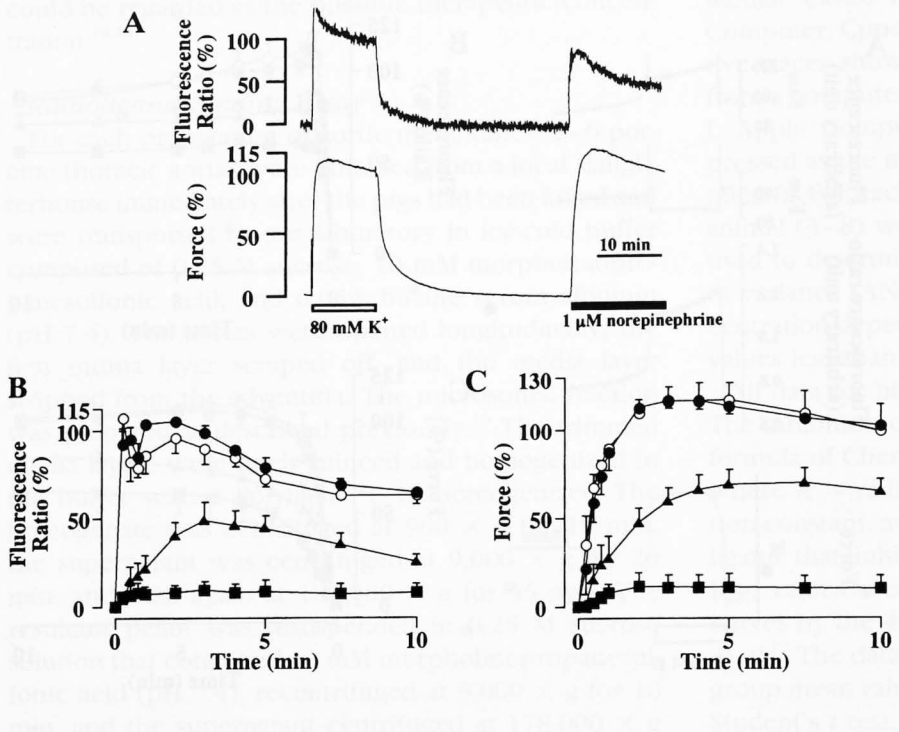

Fig. 2. Effect of chlorpromazine on the increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and force induced by norepinephrine. A: Representative recordings showing changes in the fluorescence ratio and force development induced by 1 μM norepinephrine in normal physiologic saline solution (PSS). The elevations in $[Ca^{2+}]_i$ and developed force were expressed in percentage, assigning the values in normal (5.9 mM K^+)–PSS and steady state of 80 mM K^+-PSS to be 0 and 100%, respectively. (*B and C*) Effects of various concentrations of chlorpromazine on changes in $[Ca^{2+}]_i$ (*B*) and force development (*C*) induced by 1 μM norepinephrine in normal PSS. The strips were pretreated with 0 M (○; control), 10 nM (●), 100 nM (▲), and 1 μM (■) chlorpromazine. Chlorpromazine was applied for 10 min before and was present during the application of 1 μM norepinephrine. The abscissa scales in (*B*) and (*C*) show the time (in minutes) after the application of 1 μM norepinephrine. The mean values with SE bars are shown (n = 3–5). The number of experiments refers to the number of strips from each different animal.

on norepinephrine-induced increases in $[Ca^{2+}]_i$ and force in normal PSS. Chlorpromazine inhibited the elevation of $[Ca^{2+}]_i$ and force in a concentration-dependent manner ($P < 0.05$, by two-way ANOVA). Both the first and the second phases of the norepinephrine-induced $[Ca^{2+}]_i$ increase were suppressed in parallel. Differing from the case of contraction induced by 80 mM K^+-depolarization, chlorpromazine at the concentration of 1 μM almost completely inhibited the increases in $[Ca^{2+}]_i$ and force induced by norepinephrine (9.1 ± 6.2% for $[Ca^{2+}]_i$ and 11.6 ± 7.9% for force; n = 3). Chlorpromazine preferentially decreased the norepinephrine-induced increases in $[Ca^{2+}]_i$ and force, compared with those induced by 80 mM K^+-PSS ($P < 0.05$, by two-way ANOVA).

Effects of Chlorpromazine on the Increases in $[Ca^{2+}]_i$ and Force Induced by U46619 in Normal Physiologic Saline Solution

When 1 μM U46619 was applied in normal PSS, $[Ca^{2+}]_i$ rapidly increased to form a peak (first phase), and thereafter declined, but still remained at a higher level than the prestimulation level (second phase). In the case of U46619-induced contractions, force also developed rapidly and was maintained for at least 10 min (fig. 3A). The $[Ca^{2+}]_i$ observed at maximum and steady state level were 115.7 ± 8.0% and 63.8 ± 11.2% of those induced by high K^+-depolarization, respectively. The force observed at the second phase, 10 min after application, was equal to that at a maximum level, which accounted for 126.3 ± 6.8% of that induced by high K^+-depolarization (n = 3). Figures 3B and 3C show the effects of various concentrations of chlorpromazine on the increases in $[Ca^{2+}]_i$ and force induced by U46619 in normal PSS. Under the treatment with 1 μM chlorpromazine, the $[Ca^{2+}]_i$ observed at maximum and steady state level were 102.6 ± 18.7% and 54.3 ± 5.9% of those induced by high K^+-depolarization, respectively. The force observed at steady state level was equal to that at a maximum level, which accounted for 135.1 ± 1.5% of that induced by high K^+ depolarization (n = 3). Chlorpromazine had no significant effect on the increase in $[Ca^{2+}]_i$ and force induced by U46619 (by two-way ANOVA).

Effects of Chlorpromazine on the Increases in $[Ca^{2+}]_i$ and Force Induced by U46619 in the Absence of Extracellular Ca^{2+}

The effect of chlorpromazine on the intracellular Ca^{2+} release mechanism was determined in particular. When the strips were incubated in Ca^{2+}-free PSS that contained 2 mM EGTA, $[Ca^{2+}]_i$ gradually reduced to reach a steady state, whereas the force remained at the resting level. When 1 μM U46619 was applied at the

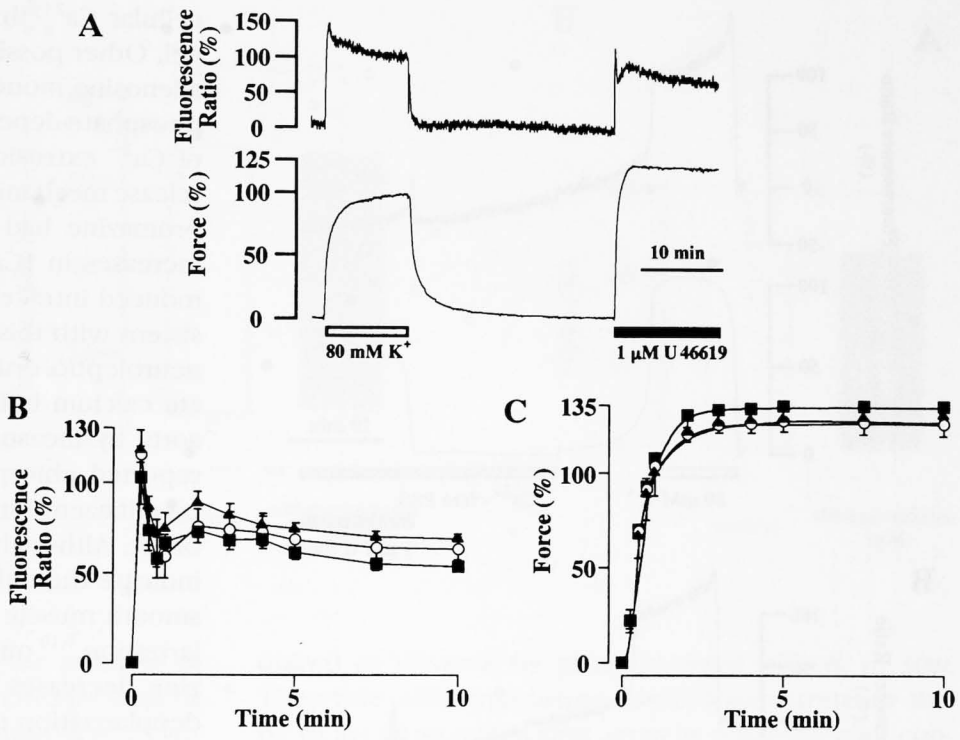

Fig. 3. Effect of chlorpromazine on the increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and force induced by U46619. (*A*) Representative recording showing changes in the fluorescence ratio and force development induced by 1 μM U46619 in normal physiologic saline solution (PSS). The elevations in $[Ca^{2+}]_i$ and developed force were expressed in percentage, in the same manner as in fig. 2A. (*B and C*) Effects of various concentrations of chlorpromazine on changes in $[Ca^{2+}]_i$ (*B*) and force development (*C*) induced by 1 μM U46619 in normal PSS. The strips were pretreated with 0 M (○; control), 100 nM (▲), and 1 μM (■) chlorpromazine. Chlorpromazine was applied 10 min before and was present during the application of 1 μM U46619. The abscissa scales in (*B*) and (*C*) show the time (in minutes) after the application of 1 μM U46619. The mean values with SE bars are shown (n = 3–5). The number of experiments refers to the number of strips from each different animal.

steady state in Ca^{2+}-free PSS, an increased elevation in $[Ca^{2+}]_i$ associated with a sustained force development occurred (fig 4A). When the strips were pretreated with 1 μM chlorpromazine in Ca^{2+}-free PSS, no significant change in $[Ca^{2+}]_i$ or force was observed (fig. 4B). Chlorpromazine at the concentration of 1 μM had no effect on the transient increases in $[Ca^{2+}]_i$ or force at the concentration of 1 μM induced by U46619 in the absence of extracellular Ca^{2+} (figs. 4B and 5, by unpaired Student's *t* test), which indicated that chlorpromazine does not inhibit the intracellular Ca^{2+} release mechanism, which is presumably mediated by inositol 1,4,5 trisphosphate.

Radioligand Binding Assay

Because the data obtained so far indicated that the potent mechanism for chlorpromazine-induced vasorelaxation would be due to the α-adrenergic blocking action, we measured the affinity of chlorpromazine to vascular α-adrenergic receptors by using [^{3}H] prazosin binding to the porcine aortic membranes. For comparison, we also investigated the α-adrenergic blocking action of a typical α-adrenergic blocking agent, phentolamine, and other neuroleptics, including trifluoperazine (another phenothiazine derivative), haloperidol (butyrophenone derivative), and imipramine (tricyclic antidepressant). In the displacement study, as shown in figure 6, chlorpromazine, haloperidol, phentolamine, trifluoperazine, and imipramine inhibited the specific binding of [^{3}H] prazosin binding to the porcine aortic membranes, in this order of potency. The slope factor and the K*i* values are shown in table 1. The slope factors of the competition curves of these compounds were near unity, which indicated that the binding was to a single population of binding sites. These data clearly showed that these neuroleptics, including chlorpromazine, bind to vascular α_1-adrenergic receptors.

Discussion

The purpose of the current study was to clarify the mechanism for the vasorelaxing effects of chlorpromazine on vascular smooth muscle. The major findings of the current study are as follows: (1) Chlorpromazine has a calcium antagonistic action, because it decreases the $[Ca^{2+}]_i$ and force of the contraction induced by high K^+-depolarization; (2) Chlorpromazine also has an α-adrenergic blocking action; (3) As a mechanism for chlorpromazine-induced vasorelaxation, the α-adrenergic blocking action plays a major role than calcium antagonistic action; (4) Chlorpromazine has almost no effect on U46619-induced contraction or U46619-in-

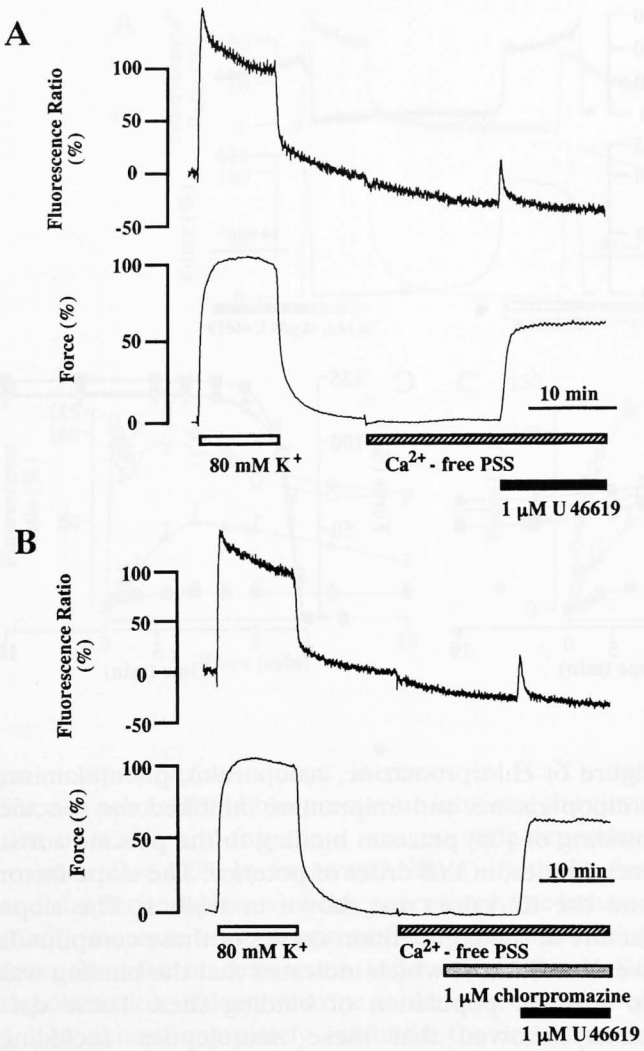

Fig. 4. Representative recordings showing changes in the fluorescence ratio and force development induced by 1 μM U46619 in Ca^{2+}-free physiologic saline solution in the absence (*A*) and presence (*B*) of 1 μM chlorpromazine. Chlorpromazine was applied 10 min before and was present during the application of 1 μM U46619. Note that chlorpromazine had no effect on the increase in intracellular Ca^{2+} concentration and force induced by 1 μM U46619.

duced intracellular Ca^{2+} release, which indicates that it has a negligible effect on the intracellular signal transduction; (5) Several neuroleptics, including chlorpromazine, induce an inhibition of [^{3}H] prazosin binding to vascular α_1-adrenergic receptors.

Chlorpromazine suppressed the increases in $[Ca^{2+}]_i$ and force induced by 80 mM K^+-depolarization in a concentration-dependent manner (figs. 1B and 1C). The most plausible explanation for this observation may be that chlorpromazine inhibits the influx of extracellular Ca^{2+} through a voltage-dependent Ca^{2+} channel. Other possibilities, such as the activation of cyclic adenosine monophosphate or cyclic guanosine monophosphate-dependent protein kinases, the acceleration of Ca^{2+} extrusion, and inhibition of intracellular Ca^{2+} release mechanisms, could be ruled out, because chlorpromazine had almost no effect on U46619-induced increases in $[Ca^{2+}]_i$ (figs. 3B and 3C) or on U46619-induced intracellular Ca^{2+} release (figs. 4 and 5). Consistent with this conclusion, Flaim *et al.*[9] reported that neuroleptic drugs, including chlorpromazine, attenuate calcium influx and tension development in rabbit aorta by measuring ^{45}Ca influx rate. Schaeffer *et al.*[10] reported chlorpromazine had high affinity for [^{3}H]d-cis-diltiazem binding site on rat cerebral cortex membrane. Although few reports have been published that indicate the chlorpromazine-induced inhibition of the smooth muscle contraction induced by high K^+-depolarization,[9,10] our data directly showed that chlorpromazine decreases $[Ca^{2+}]_i$ during activation by high K^+-depolarization in intact vascular smooth muscle.

Chlorpromazine inhibited the increases in $[Ca^{2+}]_i$ and force induced by norepinephrine in a concentration-dependent manner (figs. 2B and 2C). The site of action of chlorpromazine-induced inhibition for the norepinephrine-induced contraction is located on the receptor agonist association process, but not on the intracellular signal transduction system, because chlorpromazine had little effect on the U46619-induced contraction in the presence or absence of extracellular Ca^{2+} (Figs. 3, 4, and 5). In vascular smooth muscle, norepinephrine and U46619 share much of the intracellular signal transduction systems. The conclusion obtained by this pharmacologic study was confirmed by the biochemical study (*i.e.,* radioligand binding study). We concluded, therefore, that chlorpromazine has an α-adrenergic blocking action.

As to the relative importance of the calcium antagonistic action and an α-adrenergic blocking action for the chlorpromazine-induced vasorelaxation, the current results indicated that the α-adrenergic blocking action might play a major role, because the calcium antagonistic action may require a much higher concentration of chlorpromazine than the α-adrenergic blocking action. The therapeutic blood concentrations of chlorpromazine clinically used range from 30–350 ng/ml for adults and 40–80 ng/ml for children.[14,15] These concentrations are approximately 100 nM–1 μM. Considering the fact that chlorpromazine highly binds to protein,[20] the concentrations less than 100 nM

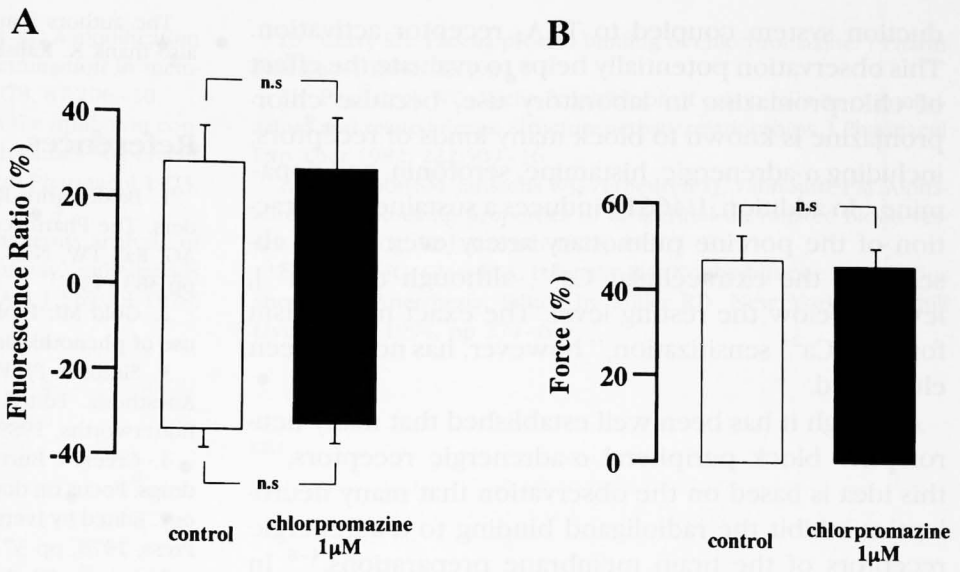

Fig. 5. Effects of 1 μM chlorpromazine on changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (*A*) and force development (*B*) induced by 1 μM U46619 in Ca^{2+}-free physiologic saline solution. A summary of the experiments done in the same manner as in fig. 4. Open and solid columns show the maximum responses induced by the application of U46619 in Ca^{2+}-free physiologic saline solution in the presence and absence of chlorpromazine, respectively. The bottom and top of the column indicate the levels before the stimulation and peak response after the stimulation by U46619, respectively. The mean values with SE bars are shown (n = 7 for the control and 8 for those with chlorpromazine). The number of experiments refers to the number of strips from each different animal. ns = not significant.

in the current study could, therefore, be possible *in vivo*. As shown in the results, 100 nM chlorpromazine inhibited norepinephrine-induced contraction to approximately 50% of the control (fig. 2), although it showed less of an effect in suppressing the K^+-induced contraction (fig. 3). In addition, because we used a higher concentration of norepinephrine to stimulate the effect in pulmonary arterial strips, such a higher concentration of chlorpromazine might also be required to observe its pharmacologic effects *in situ*. Therefore, chlorpromazine could demonstrate an ability to act as an α-blocking agent at relatively high concentrations within the therapeutic range. At higher concentrations, calcium antagonistic property might also play a role in the induction of hypotension *in vivo*. In addition, although several neuroleptics are known as calmodulin antagonists pharmacologically, the concentrations needed to inhibit calmodulin activity is more than 10 μM, which is far higher than the available therapeutic concentrations.[21] Therefore, the anticalmodulin activity, if any, may have little effect on the mechanism of neuroleptics-induced vasorelaxation.

Chlorpromazine has almost no effect on U46619-induced contraction or U46619-induced intracellular Ca^{2+} release, which indicates that chlorpromazine does not block TXA_2 receptor or intracellular signal trans-

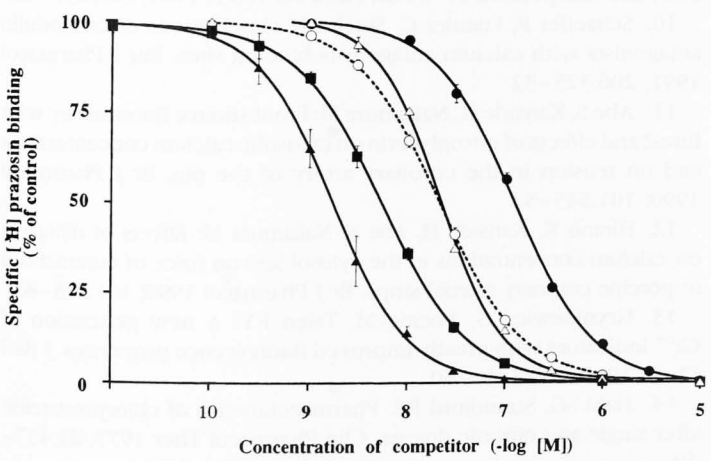

Fig. 6. Competition for [^{3}H] prazosin binding by phentolamine and selected neuroleptics. Various concentrations of chlorpromazine (▲), haloperidol (■), phentolamine (○), trifluoperazine (△), and imipramine (●) were incubated with aortic membranes and [^{3}H] prazosin (0.1 nM) for 30 min at 25°C. The values are the means ± SE of 3–5 experiments determined in duplicate or triplicate, from 3–5 different preparations of membranes. On the vertical axis, the data are expressed as percentage of control-specific [^{3}H] prazosin binding.

Table 1. The Slope Factors and the K_i Values of the Competition Curves for [^{3}H]Prazosin Binding to α-Adrenergic Receptor by Phentolamine and Other Selected Neuroleptics

Compound	n	K_i* (nM)		Slope Factor
Chlorpromazine	4	0.73 ± 0.11		1.11 ± 0.07
Haloperidol	3	2.20 ± 0.19		0.96 ± 0.03
Phentolamine	5	8.51 ± 0.52	NS	1.01 ± 0.02
Trifluoperazine	3	9.30 ± 0.23		1.32 ± 0.03
Imipramine	3	52.1 ± 3.7		1.09 ± 0.63

NS = not significant.

*K_i values of drugs were compared with each other by unpaired Student's *t* test and a significant difference was observed between all pairs ($P < 0.05$), except for between phentolamine and trifluoperazine.

duction system coupled to TXA_2 receptor activation. This observation potentially helps to evaluate the effect of chlorpromazine in laboratory use, because chlorpromazine is known to block many kinds of receptors, including α-adrenergic, histamine, serotonin, and dopamine.[7] In addition, U46619 induces a sustained contraction of the porcine pulmonary artery even in the absence of the extracellular Ca^{2+}, although the $[Ca^{2+}]_i$ level is below the resting level. The exact mechanism for this "Ca^{2+} sensitization," however, has not yet been elucidated.

Although it has been well established that many neuroleptics block peripheral α-adrenergic receptors,[1,22] this idea is based on the observation that many neuroleptics inhibit the radioligand binding to α-adrenergic receptors of the brain membrane preparations.[4-8] In the current study, we showed, for the first time, that several neuroleptics induce the inhibition of [^{3}H] prazosin binding to vascular α_1-adrenergic receptors (fig. 6 and table 1). Among the several neuroleptics tested, chlorpromazine showed the highest affinity for α_1-adrenergic receptors, which was even higher than that of typical α-blocker, phentolamine. These results are in agreement with those of a previous study done in the brain membrane preparations.[7] Because one of the derivatives of butyrophenone, haloperidol, also has a high affinity for vascular α_1-adrenergic receptors, and is used in neurolept analgesia,[23] it, too, may cause symptomatic hypotension, as seen with phenothiazines. In addition, the α-adrenergic blocking action of trifluoperazine also should be taken into account, when it was used as a calmodulin antagonist in laboratory use. However, it should be noted that there was a discrepancy between the K_i value obtained by the binding study and the potency of chlorpromazine-induced inhibition of contraction. This discrepancy could be explained by the differences in the affinity constants obtained by the binding study in the cell-free *in vitro* experiments at 25°C and those obtained by the *in situ* functional study at 37°C.

In summary, we examined the direct effects of chlorpromazine on vascular smooth muscle. Chlorpromazine caused vasorelaxation through an α-adrenergic blocking action as well as a calcium antagonistic action, and the former action is considered as having a major role in this vasorelaxation. We provided, therefore, for the first time, evidence that many neuroleptics, including chlorpromazine, strongly block vascular α_1-adrenergic receptors.

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References

1. Baldessarini RJ: Drugs and the treatment of psychiatric disorders, The Pharmacological Basis of Therapeutics. Edited by Gilman AG, Rall TW, Nies AS, Taylor P. New York, Pergamon Press, 1990, pp 383-435

2. Gold MI: Profound hypotension associated with preoperative use of phenothiazines. Anesth Analg 1974; 53:844-8

3. Simpson PJ: Pharmacological methods of vasodilation, General Anesthesia. Edited by Nunn JF, Utting JE, Brown BR Jr. London, Butterworths, 1989, pp 562-4

4. Creese I, Burt D, Snyder SH: Biochemical actions of neuroleptic drugs: Focus on dopamine receptor, Handbook of Psychopharmacology. Edited by Iversen LL, Iversen SD, Snyder SH. New York, Plenum Press, 1978, pp 37-89

5. Janssen PA, Van Bever WF: Preclinical psychopharmacology of neuroleptics, Principles of Psychopharmacology. Edited by Clark WG, del Guidice J. New York, Academic Press, 1978, pp 279-95

6. Snyder SH, U'Prichard D, Greenberg DA: Neurotransmitter receptor binding in the brain, Psychopharmacology: A Generation of Progress. Edited by Lipton MA, DiMascio A, Killam KF. New York, Raven Press, 1978, pp 361-70

7. Peroutka SJ, Snyder SH: Relationship of neuroleptic drug effects at brain dopamine, serotonin, α-adrenergic, and histamine receptors to clinical potency. Am J Psychiatry 1980; 137:1518-22

8. Cohen BM, Lipinski JF: In vivo potencies of antipsychotic drugs in blocking alpha 1 noradrenergic and dopamine D2 receptors: Implications for drug mechanisms of action. Life Sci 1986; 39:2571-80

9. Flaim SF, Brannan MD, Swigart SC, Gleason MM, Muschek LD: Neuroleptic drugs attenuate calcium influx and tension development in rabbit thoracic aorta: Effects of pimozide, penfluridol, chlorpromazine, and haloperidol. Proc Natl Acad Sci U S A 1985; 82:1237-41

10. Schaeffer P, Lugnier C, Stoclet JC: Interactions of calmodulin antagonists with calcium antagonists binding sites. Eur J Pharmacol 1991; 206:325-32

11. Abe S, Kanaide H, Nakamura M: Front-surface fluorometry with fura-2 and effects of nitroglycerin on cytosolic calcium concentrations and on tension in the coronary artery of the pig. Br J Pharmacol 1990; 101:545-52

12. Hirano K, Kanaide H, Abe S, Nakamura M: Effects of diltiazem on calcium concentrations in the cytosol and on force of contractions in porcine coronary arterial strips. Br J Pharmacol 1990; 101:273-80

13. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260:3440-50

14. Dahl SG, Strandjord RE: Pharmacokinetics of chlorpromazine after single and chronic dosage. Clin Pharmacol Ther 1977; 21:437-48

15. Rivera-Calimlim L, Nasrallah H, Strauss J, Lasagna L: Clinical response and plasma levels: Effect of dose, dosage schedules, and drug interactions on plasma chlorpromazine levels. Am J Psychiatry 1976; 133:646-52

16. Nishimura J, Kanaide H, Nakamura M: Binding of [^{3}H]prazosin to porcine aortic membranes: Interaction of calcium antagonists with vascular alpha-1 adrenoceptors. J Pharmacol Exp Ther 1986; 236:789-93

17. Markwell MAK, Haas SM, Bieber LL, Tolbert NE: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978; 87:206-10

18. Cheng Y, Prusoff W: Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 1973; 22:3099-108

19. DeLean A, Manson PJ, Rodbard D: Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligands assay, and physiological dose response curves. Am J Physiol 1978; 235:E97-102

20. Curry SH: Plasma protein binding of chlorpromazine. J Pharm Pharmacol 1970; 22:193-7

21. Prozialeck WC, Weiss B: Inhibition of calmodulin by phenothiazines and related drugs: Structure-activity relationships. J Pharmacol Exp Ther 1982; 222:509-16

22. Muldoon SM, Janssens WJ, Verbeuren TJ, Vanhoutte PM: Alpha-adrenergic blocking properties of droperidol on isolated blood vessels of the dog. Br J Anaesth 1977; 49:211-6

23. Reves JG, Glass PSA, Lubarsky DA: Nonbarbiturate intravenous anesthesia, Anesthesia. Edited by Miller RD. New York, Churchill Livingstone, 1994, pp 274-6