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(±)-Tamsulosin, an α_{1A}-adrenoceptor antagonist, inhibits the positive inotropic effect but not the accumulation of inositol phosphates in rabbit heart

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Abstract

The influence of (±)-tamsulosin, a selective α_{1A}-adrenoceptor antagonist, on the positive inotropic effect and the accumulation of inositol phosphates that are induced via α_1-adrenoceptors was studied in comparison with that of another α_{1A}-adrenoceptor ligand oxymetazoline in the rabbit ventricular myocardium. Phenylephrine elicited a concentration-dependent positive inotropic effect via α_1-adrenoceptors in the presence of either (±)-bupranolol or S(−)-timolol. The mode of antagonism induced by (±)-tamsulosin on the effect of phenylephrine was dependent on the concentration applied: (±)-tamsulosin at 1 and 3 nM acted in a competitive manner, the slope of the regression line of the Schild plot being unity and the pA_2 value being 9.12; at 10 nM, it shifted further the concentration-response curve to the right without affecting the maximal response but the slope became less than unity. At 100 nM and higher, it suppressed the maximal response to phenylephrine. (±)-Tamsulosin effectively antagonized the positive inotropic effect of phenylephrine even after inactivation of α_{1B}-adrenoceptors by treatment with chlorethylclonidine, which is an indication that the (±)-tamsulosin-sensitive subtype belongs to a class resistant to chlorethylclonidine. (±)-Tamsulosin, over the range of concentrations at which it antagonized the positive inotropic effect mediated by α_1-adrenoceptors, did not affect the accumulation of [^{3}H]inositol phosphates that was induced by 10 μM phenylephrine. Oxymetazoline antagonized the positive inotropic effect of phenylephrine in a competitive manner without affecting the accumulation of inositol monophosphate induced by phenylephrine. These results indicate that the positive inotropic effect, mediated via (±)-tamsulosin- and oxymetazoline-sensitive subtype of α_1-adrenoceptors, is exerted by a subcellular mechanism that is independent of the accumulation of inositol phosphates.

Keywords: (±)-Tamsulosin [(±)-5-[2-[[2-(2-ethoxyphenoxy)ethyl]-amino]propyl]-2-methoxybenzenesulfonamide-HCl]; α_1-Adrenoceptor; Positive inotropic effect; Phosphoinositide hydrolysis; Oxymetazoline; Ventricular myocardium, rabbit

1. Introduction

Myocardial α_1-adrenoceptors mediate the positive inotropic effect of sympathomimetic amines in most mammalian species, for example, the rabbit, rat, cat, ferret and human (Scholz, 1980; Endoh, 1982; Terzic et al., 1993). Inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol, products of the acceleration of the hydrolysis of phosphoinositide that is induced by stimulation of α_1-adrenoceptors, have been postulated to play a critical role as intracellular messengers in α_1-mediated signal transduction in the mammalian heart (Brown et al., 1985; Poggioli et al., 1986; Otani et al., 1988; Scholz et al., 1988; Endoh et al., 1991). However, the role of this pathway in mediating the positive inotropic effect of α_1-adrenoceptor agonists remains controversial.

α_1-Adrenoceptors can be divided into at least two pharmacologically distinct subtypes, α_{1A}- and α_{1B}-adrenoceptors. α_{1A}-Adrenoceptors may be coupled to the acceleration of the hydrolysis of phosphoinositide and/or to L-type Ca^{2+} channels and may have a higher affinity than the α_{1B}-subtype for antagonists such as WB 4101 {*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine}, 5-methylurapidil and (+)-niguldipine. The α_{1B}-adrenoceptors are predominantly coupled to the acceleration of the hydrolysis of phosphoinositide and they are

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irreversibly blocked by chlorethylclonidine, an alkylating agent (Han et al., 1987; Minneman, 1988; Terzic et al., 1993). Although both α_{1A}- and α_{1B}-adrenoceptors are present in myocardial cells, the relative contribution of α_{1A}- and α_{1B}-adrenoceptors to the hydrolysis of phosphoinositide in the heart is not yet fully understood. Our previous studies with the subtype-selective antagonist chlorethylclonidine suggest that about 63% of α_1-adrenoceptors in the rabbit ventricular myocardium are α_{1B}-adrenoceptors (Takanashi et al., 1991) and, moreover, there seems to be a very close correlation between the extent of inhibition by chlorethylclonidine of the positive inotropic effect and the accumulation of [^{3}H]IP$_1$ (inositol monophosphate) or [^{3}H]IP$_3$ via stimulation of α_1-adrenoceptors (Takanashi et al., 1991; Yang and Endoh, 1994). By contrast, (+)-niguldipine has only a limited inhibitory effect on the positive inotropic effect and it does not influence the accumulation of inositol monophosphate, while WB 4101 shifts the concentration-response curve for phenylephrine, with partial inhibition of the acceleration of the accumulation of inositol phosphates (Endoh et al., 1992a,b; Yang and Endoh, 1994). These findings suggest that the α_{1A}-adrenoceptors in rabbit ventricular myocardium might be heterogeneous.

In recent years, molecular cloning of α_1-adrenoceptors from a variety of mammalian species has revealed the existence of three genes encoding distinct subtypes, α_{1b}, α_{1c} and α_{1d} (Lomasney et al., 1991; Hirasawa et al., 1993; Rokosh et al., 1994). The cloned α_{1b}-adrenoceptors display a pharmacology similar to that of the originally defined α_{1B}-subtype, while the cloned α_{1c}-adrenoceptors may be the same as the pharmacologically defined α_{1A}-subtype (Ford et al., 1994) and are referred to as α_{1a}-adrenoceptors (Hieble et al., 1995). Hence, a distinction between the cloned subtypes (α_{1a}, α_{1b} and α_{1d}) and the classical pharmacologically defined subtypes (α_{1A} and α_{1B}) is made by use of lower and upper case subscript throughout this study.

Tamsulosin, 5-[2-[[2-(2-ethoxyphenoxy)ethyl]-amino]propyl]-2-methoxybenzenesulfonamide-HCl, has been reported to be an extremely potent and highly selective antagonist of α_1-adrenoceptors (Honda et al., 1985) and it antagonizes the α_1-mediated positive inotropic effect with high affinity, similar to that of prazosin, in the rabbit myocardium (Hiramoto et al., 1988). In receptor-binding assays, tamsulosin has been shown to have high affinity for α_{1A}-adrenoceptors (Hanft et al., 1989; García-Sáinz et al., 1995). In addition, among cloned subtypes of α_1-adrenoceptors from the rat, this compound has a high selectivity for α_{1a}-adrenoceptors (Michel and Insel, 1994) and adrenoceptors of this type are known to exist in myocardial cells (Hirasawa et al., 1993; Rokosh et al., 1994). It is also reported that oxymetazoline, another α_1-adrenoceptor ligand (Schümann and Endoh, 1976), has selective affinity for the α_{1A}- (Faure et al., 1994) or α_{1a}-adrenoceptor (Faure et al., 1994; Minneman et al., 1994; Schwinn et al., 1995). So, we carried out the present experiments to examine whether the antagonistic action of tamsulosin on the positive inotropic effect that is mediated by α_1-adrenoceptors might be associated with inhibition of the accumulation of inositol phosphates, in comparison with another α_{1A}-adrenoceptor ligand oxymetazoline, in the rabbit ventricular myocardium.

2. Materials and methods

2.1. Analysis of inotropic effects in isolated rabbit papillary muscle

Male albino rabbits (1.8–2.2 kg) were anaesthetized with pentobarbital sodium (50 mg/kg i.v.) and then two or three papillary muscles were excised from the right ventricle of each rabbit. Muscles were mounted in 20-ml organ baths that contained Krebs-Henseleit solution (with 0.057 mM ascorbic acid and 0.027 mM EDTA · 2Na to prevent autoxidation of the compounds examined). The solution was bubbled with 95% O_2 and 5% CO_2 at 37°C (pH 7.4). The concentrations (mM) of the various components of the solution were as follows: Na^+, 142.9; K^+, 5.9; Mg^{2+}, 1.2; Ca^{2+}, 2.5; $H_2PO_4^-$, 1.2; HCO_3^-, 24.9; SO_4^{2-}, 1.2; Cl^-, 127.8; and glucose, 11.1. The muscles were stimulated electrically by square-wave pulses of 5-ms duration at a voltage about 20% above threshold and a frequency of 1 Hz. During the equilibration period (60 min) the muscles were initially stretched under a tension of 5 mN and the length was then adjusted to give 90% of the maximal developed tension. The dimensions of the papillary muscles used were as follows: length, 5.06 ± 0.07 mm; cross-sectional area 0.55 ± 0.02 mm^2 (n = 137).

In all experiments, a β-adrenoceptor antagonist (±)-bupranolol or S(−)-timolol (1 μM each) was included in the buffer to avoid any possible modulation of responses by released noradrenaline and to ensure that the response to phenylephrine did not involve activation by β-adrenoceptors. At the beginning of each experiment, phenylephrine (3 μM) was applied at least twice until reproducible responses were obtained. The concentration-response curve for phenylephrine was determined for each preparation by cumulative addition of the drug. Each succeeding concentration was added only after the preparation had achieved a steady-state response to the previous concentration. After determination of the control concentration-response curve for phenylephrine, the drug was washed out for at least 120 min and then (±)-tamsulosin (1 nM–1 μM) or oxymetazoline (0.3–10 μM) was allowed to act for 30 min before the concentration-response curve for phenylephrine was determined again. In some experiments, preparations were treated with 10 μM chlorethylclonidine for 30 min with subsequent washing for 30 min, and then the concentration-response curve for phenylephrine was determined as described above. At the end of each experi-

ment, the maximum contractile force was determined for each muscle by administration of isoprenaline (0.1 μM–0.1 mM). The response to phenylephrine was expressed as a percentage of the maximal response to isoprenaline.

2.2. Quantitation of inositol phosphate

The heart was quickly removed from a rabbit under anaesthesia with pentobarbital sodium and placed in Krebs-Henseleit solution, bubbled with 95% O_2 and 5% CO_2, at 37°C in order to wash out the blood. The experimental procedure was the same as that described previously (Takanashi et al., 1991). Slices of ventricular muscle (0.5 mm thick) were prepared with a tissue slicer (Arthur H. Thomas, Philadelphia, PA) in cold (~4°C) Krebs-Henseleit solution. After being weighed, the slices were equilibrated in Krebs-Henseleit solution for 30 min at 37°C and then the slices were preincubated with 10 μCi/ml myo-[^{3}H]inositol in Krebs-Henseleit solution for 120 min. After the preincubation, slices were washed with a fresh solution containing 5 mM myo-inositol and 10 mM LiCl and all the experiments were performed in the Li^+-containing solution. (±)-Bupranolol (1 μM) was added to the solution 20 min prior to administration of the agonist to avoid any interference due to activation of β-adrenoceptors. (±)-Tamsulosin or oxymetazoline was allowed to act for 30 min before and during administration of 10 μM phenylephrine. Phenylephrine was allowed to act for 5 min ((±)-tamsulosin-treated group) or 30 min (oxymetazoline-treated group) and then the slices were quickly blotted and put into 1.0 ml of a mixture of chloroform, methanol and 12 N HCl (100:200:1, v/v) to terminate the reaction. After addition of 0.2 ml of 5 mM EDTA, the tissue was homogenized with a homogenizer (Polytron PT-10; Kinematica, Lucerne, Switzerland). The tip of the homogenizer was rinsed with 0.5 ml of a mixture of chloroform, methanol, 12 N HCl and 5 mM EDTA (100:200:1:80, v/v), and the rinsing fluid was added to the original solution. Chloroform (0.4 ml) and 5 mM EDTA (0.5 ml) were added sequentially and the samples were centrifuged at 1400 × g for 20 min to separate the aqueous and organic phases. An aliquot of the aqueous layer was applied to a column that contained a 50% slurry of AG1-X8 (anion-exchange resin; 100–200 mesh; formate form; Bio-Rad, Richmond, CA). The column was washed first with 20 ml of distilled water and then glycerophosphoryl esters were eluted with 8 ml of a solution of 5 mM sodium tetraborate and 60 mM sodium formate (Berridge et al., 1983). Aliquots of the eluate were monitored for radioactivity in a scintillation mixture (ACS-II; Amersham, Arlington Heights, IL) with a scintillation counter (TRI-CARB 1500, Packard, Downers Grove, IL) at a counting efficiency of 66%. [^{3}H]IP_1, [^{3}H]IP_2 (inositol 1,4-bisphosphate) and [^{3}H]IP_3 were separately collected, and the radioactivity of each was quantitated. [^{3}H]IP_1 was

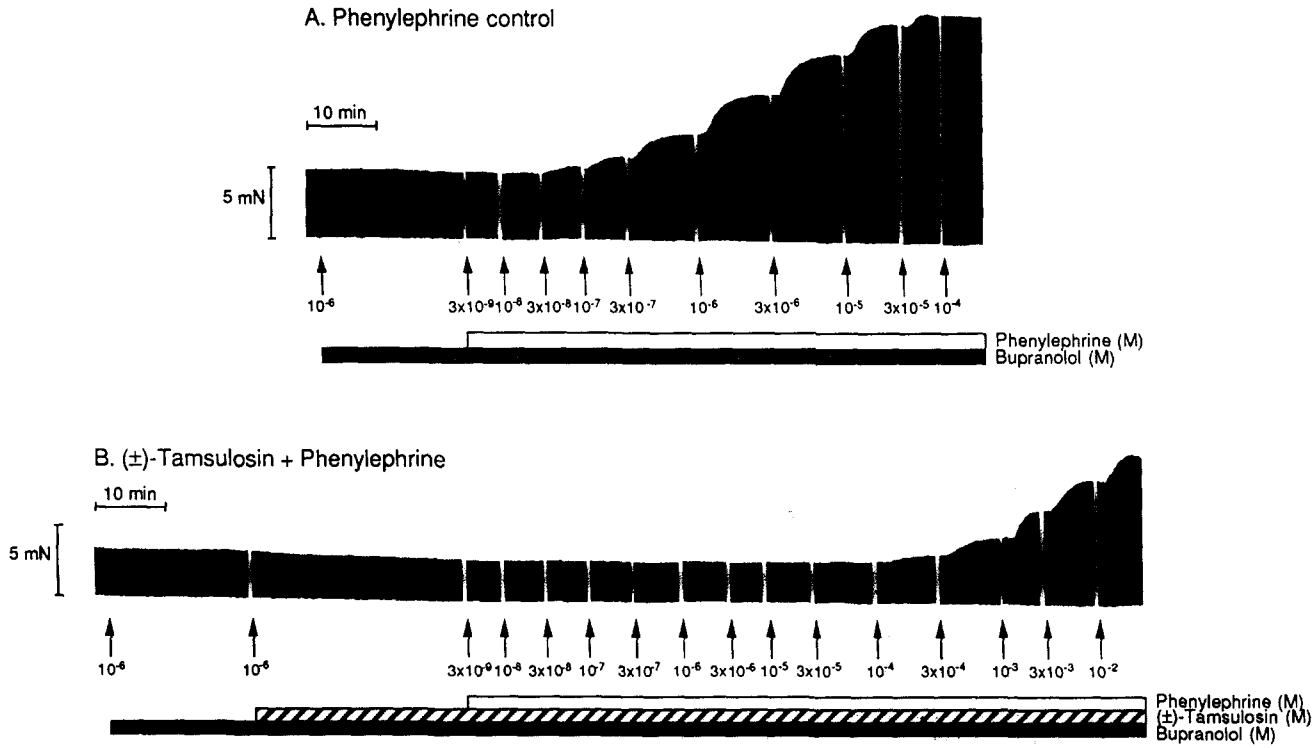

Fig. 1. Influence of 1 μM (±)-tamsulosin on the basal force of contraction and on the positive inotropic effect of phenylephrine in the presence of 1 μM bupranolol in isolated rabbit papillary muscle (1 Hz, 37°C). A: phenylephrine control; B: phenylephrine in the presence of (±)-tamsulosin. See text for details.

mainly used as an indicator of the extent of hydrolysis of phosphoinositide.

2.3. Statistical analysis

Experimental values are presented as means ± S.E. In experiments to measure the force of contraction, EC_{50} values were obtained from an analysis of individual concentration-response curves. Characteristics of pharmacological antagonism were analysed by the method of Arunlakshana and Schild (1959). Significance of differences were estimated by one-way analysis of variance (ANOVA) with Duncan's multiple range test and/or by Student's *t*-test where appropriate. A P value of < 0.05 was considered to be significant.

2.4. Drugs

The drugs used were (±)-tamsulosin [(±)-5-[2-[[2-(2-ethoxyphenoxy)ethyl]-amino]propyl]-2-methoxybenzenesulfonamide-HCl; Yamanouchi, Tokyo, Japan]; (−)-phenylephrine hydrochloride, (−)-isoprenaline hydrochloride, oxymetazoline hydrochloride, lithium chloride, myo-inositol (Sigma, St. Louis, MO, USA); ammonium formate (Wako Pure Chemical, Osaka, Japan); (±)-bupranolol hydrochloride (Kaken Pharmaceutical, Tokyo, Japan); chlorethylclonidine dihydrochloride, S(−)-timolol maleate (Research Biochemicals, Natick, MA); pentobarbital sodium (Abbott Laboratories, North Chicago, IL); myo-[2-^{3}H]inositol (specific activity 86 Ci/mmol; Amersham, Buckinghamshire, UK).

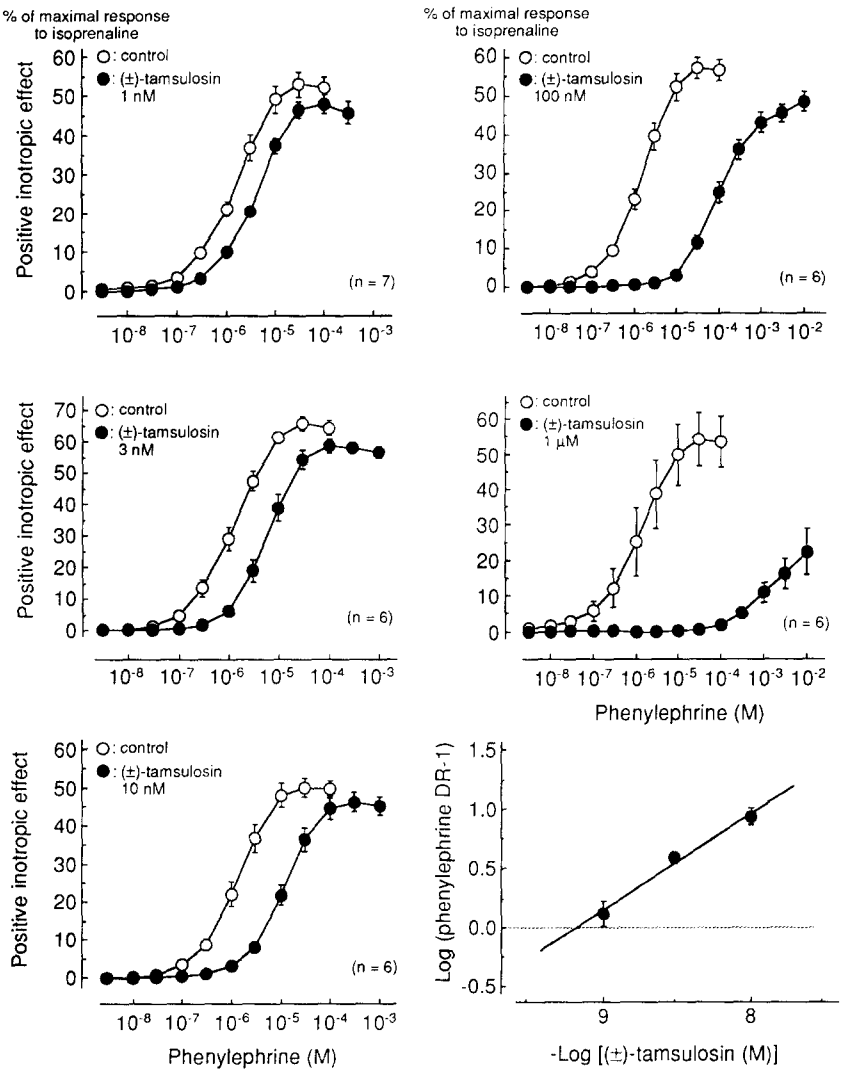

Fig. 2. Effects of (±)-tamsulosin on the concentration-response curve for the phenylephrine-induced positive inotropic effect (left and upper, middle right panels), and Schild plot of (±)-tamsulosin-induced antagonism against the effect of phenylephrine (lower right panel) mediated by α_1-adrenoceptors, in the presence of 1 μM bupranolol, in isolated rabbit papillary muscle (1 Hz, 37°C). Values presented are means ± S.E. Numbers of experiments are presented in parentheses. The basal force of contraction before addition of phenylephrine was 6.51 ± 0.76 mN/mm^2; the maximum force determined with isoprenaline was 24.5 ± 2.5 mN/mm^2 ($n = 31$). The slope of the regression line calculated by the least-squares method was 0.82 ($r = 0.99$; $n = 19$).

3. Results

3.1. Influence of (±)-tamsulosin on the α_1-adrenoceptor-mediated positive inotropic effect

The influence of (±)-tamsulosin on the positive inotropic effect of phenylephrine administered in a cumulative manner in the presence of 1 μM bupranolol is shown in Fig. 1. (±)-Tamsulosin from 1 nM to 1 μM did not significantly affect the basal force of contraction, while it antagonized the positive inotropic effect of phenylephrine in a concentration-dependent manner. (±)-Tamsulosin at 1–10 nM shifted the concentration-response curves for phenylephrine in parallel to the right without affecting the maximal response to phenylephrine, while the mode of the antagonism was different depending on the concentration of (±)-tamsulosin. The pharmacological characteristics of the (±)-tamsulosin-induced antagonism of the positive

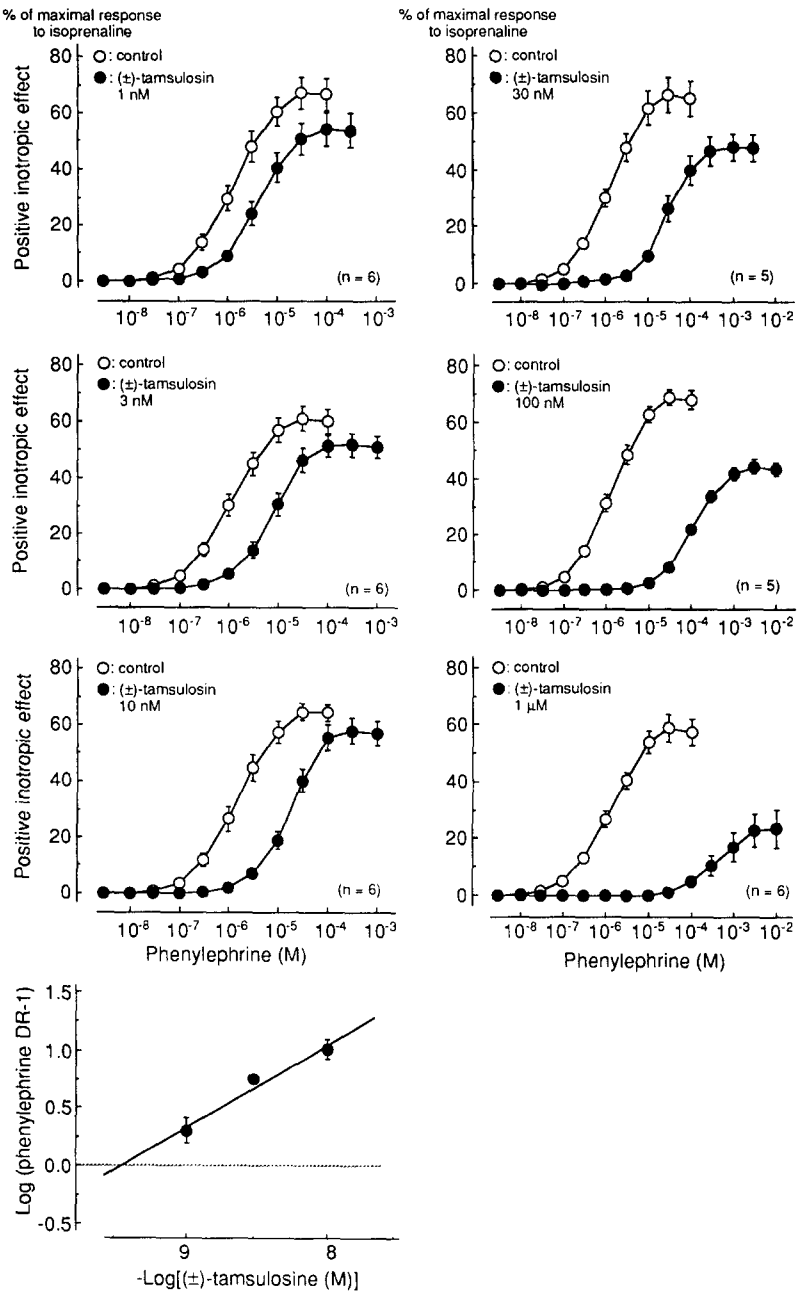

Fig. 3. Effects of (±)-tamsulosin on the concentration-response curve for the phenylephrine-induced positive inotropic effect (upper and middle panels), and Schild plot of (±)-tamsulosin-induced antagonism against the effect of phenylephrine (lowest panel) mediated by α_1-adrenoceptors, in the presence of 1 μM S(−)-timolol, in isolated rabbit papillary muscle (1 Hz, 37°C). The basal force of contraction before addition of phenylephrine was 9.78 ± 1.17 mN/mm^2; the maximum force determined with isoprenaline was 29.4 ± 5.0 mN/mm^2 (n = 34). The 213slope of the regression line calculated by the least-squares method was 0.69 (r = 0.97; n = 18).

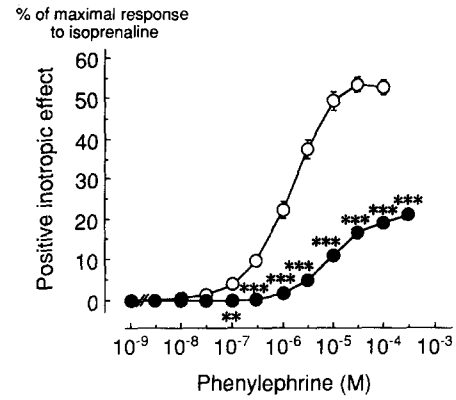

Fig. 4. Influence of treatment with chlorethylclonidine on the α_1-adrenoceptor-mediated positive inotropic effect in isolated rabbit papillary muscle (1 Hz, 37°C). The cumulative concentration-response curves for phenylephrine were determined with muscle preparations that were not treated (○; $n = 25$) or were treated with 10 μM chlorethylclonidine (●; $n = 30$) in the presence of 1 μM bupranolol. The basal force was 6.95 ± 0.75 mN/mm^2 and the maximal force determined with isoprenaline was 26.2 ± 1.9 mN/mm^2 ($n = 55$). ** $P < 0.01$; *** $P < 0.001$ vs. the corresponding control values.

inotropic effect of phenylephrine that was mediated by α_1-adrenoceptors were analysed by drawing a Schild plot. The results are shown in lowest right panels in Fig. 2. The slope of the Schild plot for concentrations of 1 and 3 nM (±)-tamsulosin was 0.99, which was not significantly different from unity, and the line intercepted the x-axis at a pA_2 value of 9.12. However, when the higher concentration of (±)-tamsulosin was included in the analysis, the slope of the Schild plot became 0.82 (Fig. 2), viz. less than unity ($P < 0.05$), indicating interference of the theoretical competitive antagonism with the contribution of the subtype that was less sensitive to (±)-tamsulosin. (±)-Tamsulosin at 100 nM and higher shifted the curves to the right and downward as shown in the upper and middle right-hand panels in Fig. 2, while there was a partial contribution of β-adrenoceptor-mediated response to very high concentrations of phenylephrine because the antagonism by bupranolol was competitive (Kohi et al., 1993a). The positive inotropic effect of high concentrations of phenylephrine observed in the presence of bupranolol and (±)-tamsulosin at 100 nM and higher was decreased in the

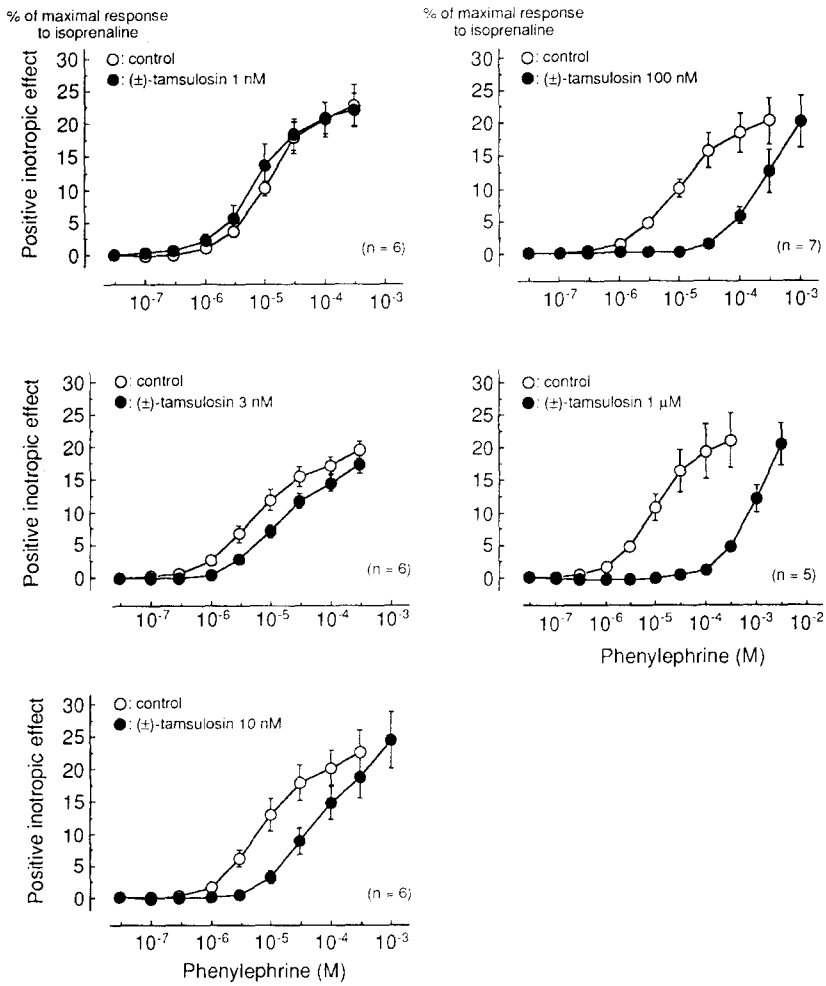

Fig. 5. Influence of (±)-tamsulosin on the positive inotropic effect of phenylephrine in muscle preparations treated with 10 μM chlorethylclonidine in the presence of 1 μM bupranolol (1 Hz, 37°C). Control in chlorethylclonidine-treated muscle (○); (±)-tamsulosin (●) in chlorethylclonidine-treated muscle. Numbers of experiments are presented in parentheses. The basal force was 7.52 ± 1.19 mN/mm^2 and the maximum force determined with isoprenaline was 27.7 ± 2.4 mN/mm^2 ($n = 30$).

presence of another potent β-adrenoceptor blocking agent, S(−)-timolol as well, implying a contribution of the β-adrenoceptor-mediated response indeed (Fig. 3).

The inhibitory action of (±)-tamsulosin in the presence of another β-adrenoceptor blocker, S(−)-timolol, 1 μM (Fig. 3), was very similar to that observed in the presence of 1 μM bupranolol. The slope of the Schild plot was 0.94 for concentrations of 1 and 3 nM (±)-tamsulosin, and the line intercepted the x-axis at a pA_2 value of 9.34. However, when the higher concentration of 10 nM (±)-tamsulosin was included in the analysis, the slope of the Schild plot became 0.69 (Fig. 3), which was less than unity ($P < 0.05$). (±)-Tamsulosin at 100 nM suppressed the maximal response to phenylephrine more prominently in the presence of timolol, indicating that timolol is more potent than bupranolol as a β-adrenoceptor blocking agent.

When the extracellular calcium concentration was decreased to 1.25 mM from 2.50 mM, the maximal inotropic response to phenylephrine was significantly decreased to 57.4 ± 1.5% ($n = 18$) vs. 64.7 ± 1.8% ($n = 34$, $P < 0.01$) of the maximal response to isoprenaline, while the basal force of contraction did not change significantly. The inhibitory action of (±)-tamsulosin (1–10 nM) in the presence of 1.25 mM extracellular calcium concentration and 1 μM timolol was essentially the same as that with the extracellular calcium concentration of 2.50 mM ($n = 18$; data not shown).

3.2. Influence of (±)-tamsulosin on the α_1-adrenoceptor-mediated positive inotropic effect in preparations treated with chlorethylclonidine

Inactivation of α_{1B}-adrenoceptors by treatment with 10 μM chlorethylclonidine did not alter the basal force of contraction, but it shifted the concentration-response curve for phenylephrine to the right and decreased its slope (Fig. 4). The maximal inotropic effect of phenylephrine was diminished by 64.4% (to 19.0 ± 1.2% of the maximal response to isoprenaline in chlorethylclonidine-treated muscles from 53.4 ± 1.9% in the control) and the EC_{50} for phenylephrine was increased 5.3-fold from a control value of 1.58 ± 0.13 μM ($n = 25$) to 8.40 ± 0.62 μM ($n = 30$) in chlorethylclonidine-treated muscles.

In muscles treated with chlorethylclonidine, (±)-tamsulosin effectively antagonized the phenylephrine-induced positive inotropic effect (Fig. 5), an indication that the (±)-tamsulosin-sensitive subtype belongs to a class that is resistant to treatment with chlorethylclonidine. Because very high concentrations of phenylephrine had to be used in chlorethylclonidine-treated muscles, the effect of phenylephrine that is mediated by β-adrenoceptors disturbed the determination of the nature of the antagonism induced by (±)-tamsulosin in chlorethylclonidine-treated muscles. Although the maximal response to phenylephrine was apparently achieved even in the presence of high concentrations of (±)-tamsulosin, this may be due to the partial contribution of the β-adrenoceptor-mediated response seen at high concentrations of phenylephrine (Fig. 5).

3.3. Influence of (±)-tamsulosin on the α_1-adrenoceptor-mediated accumulation of [^{3}H]inositol phosphates

The influence of (±)-tamsulosin on the accumulation of [^{3}H]IP_1, [^{3}H]IP_2, [^{3}H]IP_3 in response to phenylephrine

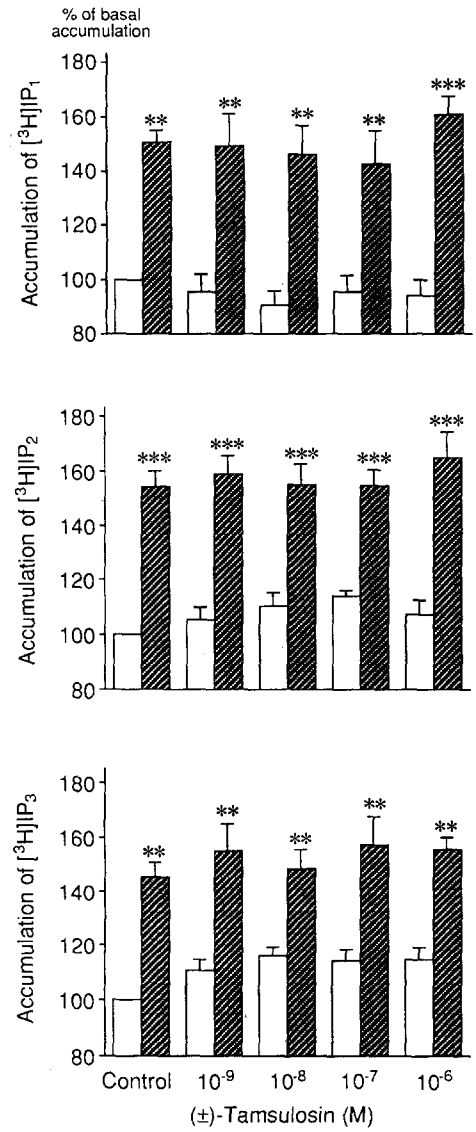

Fig. 6. Influence of various concentrations of (±)-tamsulosin on the accumulation of [^{3}H]IP_1, [^{3}H]IP_2 and [^{3}H]IP_3 induced by phenylephrine after 5 min in the presence of 1 μM bupranolol in slices of rabbit ventricular muscle. Values were determined after the administration of phenylephrine at 10 μM (striated columns) or saline (open columns). Data are expressed as a percentage of the corresponding basal control levels that were determined simultaneously (mean ± S.E., $n = 6$ each). The mean radioactivity of control slices was 29.6 ± 2.9 dpm/mg ([^{3}H]IP_1), 23.0 ± 2.7 dpm/mg ([^{3}H]IP_2) and 13.3 ± 3.1 dpm/mg ([^{3}H]IP_3), respectively. The mean wet weight of the muscle slices was 54.1 ± 1.9 mg ($n = 60$). ** $P < 0.01$; *** $P < 0.001$ vs. the corresponding values with saline.

in the presence of 1 μM bupranolol is shown in Fig. 6. Five minutes after the administration of 10 μM phenylephrine, the levels of [^{3}H]IP_1, [^{3}H]IP_2 and [^{3}H]IP_3 were increased to 150.6 ± 4.5, 154.2 ± 5.9 and 145.4 ± 5.1% of the corresponding basal level, respectively, in the absence of (±)-tamsulosin. The increases in the levels of [^{3}H]IP_1, [^{3}H]IP_2 and [^{3}H]IP_3 induced by phenylephrine were not significantly affected by (±)-tamsulosin at 1 nM–1 μM.

3.4. Influence of oxymetazoline on the α_1-adrenoceptor-mediated positive inotropic effect and accumulation of [^{3}H]inositol monophosphate

Oxymetazoline (0.3–10 μM) did not cause any inotropic effect by itself, but it antagonized the positive inotropic effect of phenylephrine in a concentration-dependent manner in the presence of 1 μM bupranolol (Fig. 7). Oxymetazoline at 0.3–1 μM shifted the concentration-response curves for phenylephrine to the right without affecting the slope or the maximal response to phenylephrine. The Schild plot yielded a linear relationship with a slope of 0.89 (not significantly different from the unity), and the line intercepted the x-axis at a pA_2 value of 6.89. The maximal response to phenylephrine was suppressed in the presence of 10 μM oxymetazoline (Fig. 7, lower right-hand panel).

The influence of oxymetazoline (0.3–10 μM) on the accumulation of [^{3}H]IP_1 in response to phenylephrine in the presence of 1 μM bupranolol is shown in Fig. 8. Thirty minutes after the administration of 10 μM phenyl-

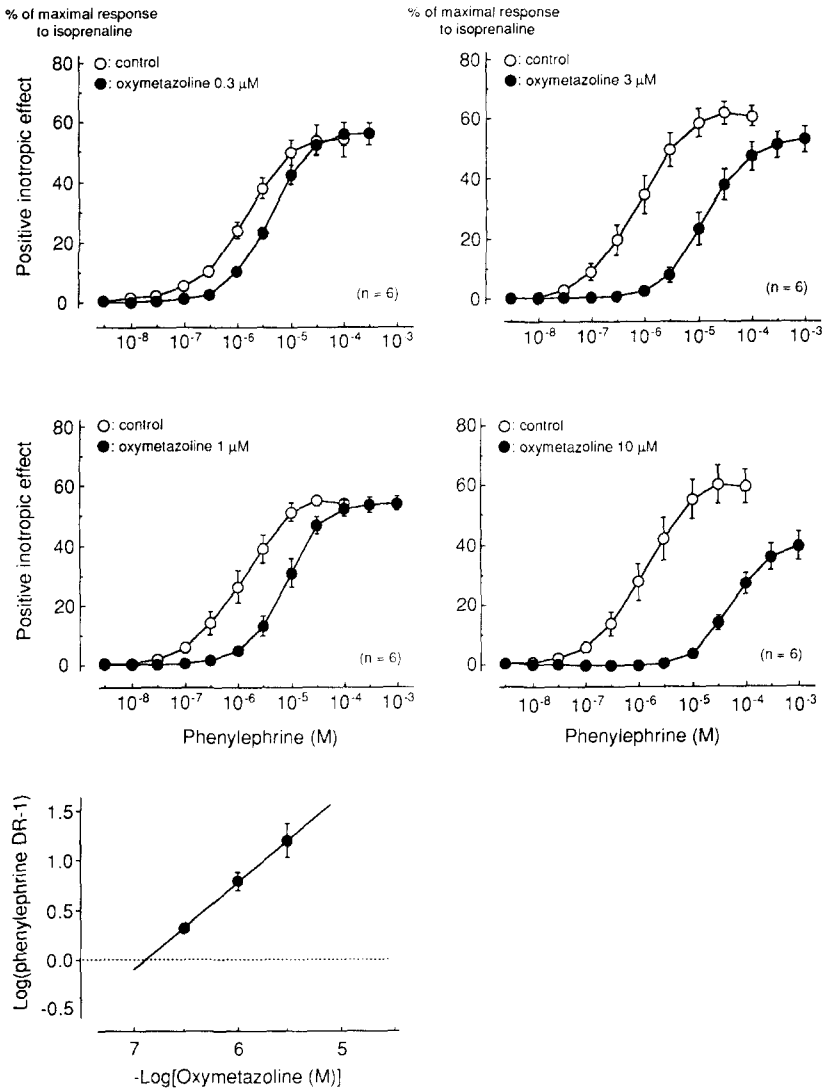

Fig. 7. Effects of oxymetazoline on the concentration-response curve for the phenylephrine-induced positive inotropic effect (upper and middle panels), and Schild plot of oxymetazoline-induced antagonism against the effect of phenylephrine (lowest panel) mediated by α_1-adrenoceptors, in the presence of 1 μM bupranolol, in isolated rabbit papillary muscle (1 Hz, 37°C). The basal force of contraction before addition of phenylephrine was 8.70 ± 1.13 mN/mm^2; the maximum force determined with isoprenaline was 29.1 ± 3.3 mN/mm^2 (n = 24, each). The lowest panel shows a Schild plot of oxymetazoline-induced antagonism against the effect of phenylephrine. The slope of the regression line calculated by the least-squares method was 0.89 (r = 0.99; n = 18).

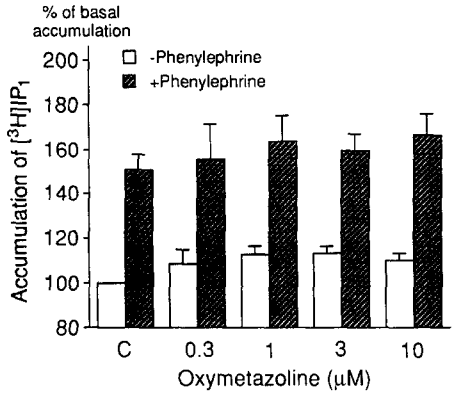

Fig. 8. Influence of various concentrations of oxymetazoline on the accumulation of inositol monophosphate ([^{3}H]IP_1) induced by 10 μM phenylephrine after 30 min in the presence of 1 μM bupranolol in slices of rabbit ventricular muscle. Values were determined after the administration of phenylephrine at 10 μM (striated columns) or saline (open columns). Oxymetazoline was allowed to act 30 min before and during the administration of phenylephrine. Data are expressed as a percentage of the corresponding basal control levels that were determined simultaneously ($n = 7$ each). The mean radioactivity of control slices was 30.3 ± 2.4 dpm/mg. The mean wet weight of the muscle slices was 64.3 ± 1.8 mg ($n = 70$).

ephrine, the level of [^{3}H]IP_1 was increased to $150.8 \pm 6.3\%$ of the basal level, which was not significantly affected by the presence of 0.3, 1, 3 and 10 μM oxymetazoline.

4. Discussion

In isolated rabbit papillary muscle, the selective α_{1A}-adrenoceptor antagonist (±)-tamsulosin (Honda et al., 1985) antagonized the positive inotropic effect of phenylephrine in a concentration-dependent manner: (±)-tamsulosin at 1 to 10 nM shifted the concentration-response curves for phenylephrine in parallel to the right without altering the maximal response, while the maximal response was attenuated at the relatively high concentrations of 100 nM and 1 μM (Figs. 1–3). This result indicates that (±)-tamsulosin acts predominantly as a competitive antagonist of α_1-adrenoceptors in rabbit ventricular muscle (Hiramoto et al., 1988).

It has been reported that (±)-tamsulosin is a selective α_{1A}-adrenoceptor antagonist on the basis of its ability to discriminate between α_{1A}- and α_{1B}-adrenoceptor recognition sites that have been labelled with [^{3}H]prazosin (Hanft et al., 1989) and [^{3}H]tamsulosin (García-Sáinz et al., 1995). This conclusion is supported by the results of Sudoh et al. (1992) who found that, after pretreatment with chlorethylclonidine of membrane fractions derived from various rabbit tissues including the heart, [^{3}H]tamsulosin exhibited affinity for the remaining binding sites greater than that of [^{3}H]prazosin or [^{3}H]WB 4101, indicating that tamsulosin has high affinity for α_{1A}-adrenoceptors. However, it has become evident that certain characteristics of the tamsulosin-induced antagonism are quite different from those of the antagonism induced by other α_{1A}-adrenoceptor antagonists. WB 4101 shifted the curve for the positive inotropic effect of phenylephrine by 1 log unit at 1 nM, whereas it did not cause a further shift at higher concentrations, probably because of the predominant contribution of α_{1B}-adrenoceptors in rabbit ventricular muscle (Endoh et al., 1992a). By contrast, (+)-niguldipine depressed the maximal response to α_1-stimulation at concentrations as low as 0.1 pM without producing a rightward shift of the concentration-response curve; this drug did not cause further inhibition at higher concentrations (Endoh et al., 1992b). (±)-Tamsulosin exhibited neither the saturation characteristics of WB 4101 nor the depression of the maximal response observed with (+)-niguldipine, so it is clearly different from the agents that have been previously recognized as selective antagonists of α_{1A}-adrenoceptors. In this context, it is noteworthy that the inhibitory action of (±)-tamsulosin was very similar to that of another α_{1A}-adrenoceptor ligand oxymetazoline (Faure et al., 1994) in respect to the mode of antagonism (Schümann and Endoh, 1976) and dissociation from the accumulation of inositol phosphate induced by phenylephrine, as will be discussed later.

The slope of the Schild plot for (±)-tamsulosin was close to unity in only a limited concentration range and it became less than unity at the higher concentration, suggesting the possible involvement of more than one subtype in the effect of phenylephrine. Because rabbit ventricular muscle contains predominantly the chlorethylclonidine-sensitive α_{1B}-subtype (Takanashi et al., 1991), we examined the possible interference of α_{1B}-adrenoceptors, by pretreatment with chlorethylclonidine, in the antagonistic action of (±)-tamsulosin. Pretreatment with chlorethylclonidine markedly diminished the maximal response and elicited a rightward shift of the concentration-response curve for phenylephrine. These results are in agreement with previous findings in papillary muscles from the rabbit (Takanashi et al., 1991; Kohi et al., 1993a) and the rat (Williamson et al., 1994). After pretreatment with 10 μM chlorethylclonidine, the EC_{50} value for phenylephrine increased 5-fold (Fig. 4). Since the relationship between occupancy of α_1-adrenoceptors and the positive inotropic effect is non-linear, indicating the existence of spare receptors in rabbit papillary muscle (Hiramoto et al., 1988), this result might explain the chlorethylclonidine-induced rightward shift of the curve for phenylephrine.

Chlorethylclonidine might also bind to subtypes other than α_{1B}-adrenoceptors. In this context, current findings imply that chlorethylclonidine is selective for α_{1B}-adrenoceptors (Han et al., 1987; Minneman et al., 1988) and that it modifies the amino acid composition of α_{1B}-adrenoceptors but not that of α_{1A}-adrenoceptors (Terman et al., 1990). However, it has recently been shown that chlorethylclonidine binds also to α_{1d}- and α_{1a}-adrenoceptors, with a rank order of $\alpha_{1b} > \alpha_{1d} > \alpha_{1a}$, when cloned adrenoceptor subtypes are examined (Goetz et al., 1993). Such an effect of chlorethylclonidine might be responsible for the

modulation of the inhibitory action of (±)-tamsulosin in muscle preparations that were pretreated with chlorethylclonidine (Fig. 5).

After treatment of preparations with chlorethylclonidine, (±)-tamsulosin effectively antagonized the positive inotropic effect of phenylephrine, indicating that the antagonistic action of (±)-tamsulosin does not involve α_{1B}-adrenoceptors. Michel and Insel (1994) reported that (±)-tamsulosin has higher affinity for α_{1a}- than for α_{1b}-adrenoceptors, whereas it has a limited ability to select between α_{1a}- and α_{1d}-adrenoceptors. Since recent pieces of evidence indicate that α_{1d} mRNA is present in the heart (Rokosh et al., 1994; Stewart et al., 1994), different subtypes that are sensitive to (±)-tamsulosin but not sensitive to chlorethylclonidine might possibly contribute to the inhibitory action of (±)-tamsulosin in the case of chlorethylclonidine-pretreated muscles (Fig. 5). (±)-Tamsulosin at a concentration of 1 μM markedly shifted the concentration-response curve for phenylephrine to the right, indicating that (±)-tamsulosin is a potent and effective α_{1A}-adrenoceptor antagonist in the rabbit ventricular myocardium. It was noted, however, in chlorethylclonidine-treated preparations that the β-adrenoceptor-mediated effect of phenylephrine appeared in the presence of high concentrations of (±)-tamsulosin and contributed partially to the maintenance of the maximal response to phenylephrine, because phenylephrine had to be applied at very high concentrations (Fig. 5).

It is noteworthy that (±)-tamsulosin at concentrations up to 1 μM did not significantly affect the accumulation of [^{3}H]IP_3 that was induced by stimulation of α_1-adrenoceptors in rabbit ventricular myocardium. This result indicates a clear dissociation of the positive inotropic effect from the accumulation of inositol phosphates. Considering the close correlation between the inotropic effect and the response in terms of accumulation of inositol phosphates during activation of α_{1B}-adrenoceptors (Takanashi et al., 1991; Yang and Endoh, 1994) and the dissociative action of (±)-tamsulosin, we are inclined to postulate that stimulation of myocardial α_{1A}-adrenoceptors produces a positive inotropic effect independent of the accumulation of inositol phosphates, in addition to a chlorethylclonidine-sensitive positive inotropic effect associated with accumulation of inositol phosphates. The previous findings in the rabbit ventricular myocardium that the antagonistic action of selective α_{1A}-adrenoceptor antagonists, such as (+)-niguldipine (Yang and Endoh, 1994), HV 723 (Kohi et al., 1993b) and WB 4101 (Yang and Endoh, 1994), on the positive inotropic effect of α_1-adrenoceptor stimulation was not or was associated with only partial inhibition of the accumulation of inositol phosphates induced by α_1-adrenoceptor stimulation support the above postulate of different mechanisms that are associated with or unrelated to the accumulation of inositol phosphates. The present findings with another α_{1A}-adrenoceptor ligand oxymetazoline (Faure et al., 1994), which antagonized the positive inotropic effect of phenylephrine mediated by α_1-adrenoceptors in a competitive manner but did not affect the accumulation of inositol monophosphate induced by phenylephrine, also support the postulate described above. In these studies, the accumulation of [^{3}H]inositol phosphates was measured in rabbit ventricular slices prelabelled with myo-[^{3}H]inositol according to the method of Berridge et al. (1983). Although accumulation of [^{3}H]inositol phosphates may reflect the activation of phospholipase C subsequent to α_1-adrenoceptor stimulation, it has been shown that myocardial α_1-adrenoceptors are coupled also to activation of phospholipase D (Ye et al., 1994) and phospholipase A_2 (Kurachi et al., 1989). Namely, activation of phospholipase D results in production of diacylglycerol, which may be shared by activation of phospholipase C. Therefore, the potential involvement of various types of phospholipase in α_1-adrenoceptor-mediated regulation of myocardial contractility has to be taken into consideration and requires future study.

In summary, we have demonstrated in rabbit ventricular muscle that: (1) (±)-tamsulosin and oxymetazoline competitively antagonize the positive inotropic effect of phenylephrine acting via a chlorethylclonidine-insensitive subtype of α_1-adrenoceptor; (2) the functional antagonism is not associated with inhibition of the accumulation of inositol phosphate induced by α_1-adrenoceptor stimulation; and, thus, (3) (±)-tamsulosin and oxymetazoline cause a clear dissociation of the positive inotropic effect from the accumulation of inositol phosphates that is induced by α_1-adrenoceptors.

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