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Methoxamine-induced inhibition of the positive inotropic effect of endothelin via α_1-adrenoceptors in the rabbit heart

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Abstract

The influence of methoxamine on the positive inotropic effect of endothelin was assessed in the isolated rabbit ventricular myocardium. Methoxamine by itself elicited a positive inotropic effect and it simultaneously inhibited the positive inotropic effects of endothelin-1 and endothelin-3 without affecting the acceleration of the hydrolysis of phosphoinositide that was induced by the endothelin isopeptides. By contrast, the positive inotropic effects induced by elevation of concentration of external Ca^{2+} ions, by Bay k 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), by dihydroouabain and by forskolin were unaffected by methoxamine. The inhibitory action of methoxamine was abolished by α_1-adrenoceptor antagonists, such as prazosin, WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and (±)-tamsulosin; and it was inhibited to a lesser extent by chlorethylclonidine. In addition, methoxamine did not modify the specific binding of [^{125}I]endothelin-3 to ventricular membrane fraction. These results indicate that methoxamine antagonizes the positive inotropic effect of endothelin isopeptides at the level of the signal-transduction process, subsequent to acceleration of the hydrolysis of phosphoinositide, via activation of α_1-adrenoceptors in the rabbit ventricular myocardium.

Keywords: Methoxamine; α_1-Adrenoceptor; Positive inotropic effect; Endothelin; Ventricular myocardium, rabbit

1. Introduction

Methoxamine has been used as a prototype of α-adrenoceptor agonists in pharmacological experiments as well as in a clinical setting. While it has been established that methoxamine acts preferentially on α-adrenoceptors, methoxamine has additional effects on β-adrenoceptors and a direct, nonspecific action on ion channels, and thus, it has stimulatory as well as inhibitory effects on cardiac contraction (for reviews, see Scholz, 1980; Endoh, 1982). In the rabbit ventricular myocardium, methoxamine stimulates the hydrolysis of phosphoinositide with an efficacy identical to that of phenylephrine, whereas the potency of methoxamine is approximately ten times lower than that of phenylephrine (Yang and Endoh, 1994). It is also remarkable that, in contrast to phenylephrine, methoxamine elicits a much less pronounced positive inotropic effect upon cumulative administration than upon single administration. Moreover, methoxamine inhibits the positive inotropic effect of phenylephrine over a range of concentrations at which it causes the acceleration of the hydrolysis of phosphoinositide (Yang and Endoh, 1994).

The present study was carried out to elucidate further details of the methoxamine-induced inhibition of the receptor-mediated positive inotropic effect. With this goal in mind, we examined the influence of methoxamine on the positive inotropic effect that is mediated by activation of endothelin receptors, which are similarly coupled to acceleration of the hydrolysis of phosphoinositide in the rabbit ventricular muscle (Takanashi and Endoh, 1991). We also investigated the influence of methoxamine on inotropic effects that are exerted independently of the hydrolysis of phosphoinositide, namely, on the effects of elevation of $[Ca^{2+}]_o$ (concentration of external Ca^{2+} ions), Bay k 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), dihydroouabain

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and forskolin in the rabbit ventricular myocardium. Furthermore, the influence of α_1-adrenoceptor antagonists was examined to determine whether specific receptors are involved in the inhibitory action of methoxamine.

2. Materials and methods

2.1. Isolated rabbit papillary muscles

Male albino rabbits (1.8–2.2 kg) were anaesthetized with pentobarbital sodium (50 mg/kg, i.v.), then the heart of each was removed and immersed immediately in Krebs-Henseleit solution that was bubbled with 95% O_2 and 5% CO_2 at 37°C. Two or three papillary muscles were excised from the right ventricle and mounted in 20 ml organ baths that contained Krebs-Henseleit solution (with 0.057 mM ascorbic acid and 0.027 mM ethylenediaminetetraacetic acid, disodium salt, 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 electrically stimulated by square-wave pulses of 5-ms duration at a voltage that was about 20% above the threshold and at a frequency of 0.5 Hz and the force of isometric contraction was measured by force displacement transducers (Shinkoh UL 10 GR, Minebea, Tokyo, Japan). During the equilibration period of 60 min, the muscles were initially stretched by a tension of 5 mN and the length was then adjusted to give 90% of the maximal contractile force. The experimental procedure has been described previously in detail (Kushida et al., 1988). Bupranolol (0.3 μM) was allowed to act for 20 min before the administration of methoxamine or phenylephrine. At the beginning of each experiment, phenylephrine at a single concentration (1 μM, in the presence of bupranolol) was administered two or three times until reproducible responses were obtained to confirm the responsiveness of the preparation to activation of receptor.

Endothelin isopeptides were administered in a cumulative manner by stepwise increases in concentrations of each agonist in log 0.5 units. Only one concentration-response curve for endothelin isopeptides was determined for each muscle preparation because the baseline level of contractile force was not achieved even after a 120-min washout of endothelin isopeptides. Methoxamine was allowed to act for 30 min before addition of endothelin-1 or endothelin-3, and it was present in the organ bath throughout the experiments. The influence of incubation with methoxamine for 15 min and 60 min on the positive inotropic effect of endothelin-3 was also determined. α_1-Adrenoceptor antagonists, prazosin, WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and (±)-tamsulosin, were added 30 min before the administration of methoxamine and during the administration of agonists. Chlorethylclonidine was administered for 30 min and was then washed out of the organ bath. After washout for 30 min, methoxamine was added. In another series of experiments, the influence of other sympathomimetic amines, noradrenaline and phenylephrine was investigated. In these experiments, noradrenaline or phenylephrine was allowed to act for 30 min and the cumulative concentration-response curves of endothelin-3 were determined in their presence. Since noradrenaline is a β-adrenoceptor full agonist, the experiments with noradrenaline were carried out in the presence of 1 μM bupranolol. In other series of experiments, after cumulative concentration-response curves for $[Ca^{2+}]_0$, dihydroouabain and forskolin have been obtained, the agents were washed out until the baseline force was restored, and then another set of concentration-response curves was generated in the presence of methoxamine. At the end of each experiment, the maximum contractile force was determined for each muscle by the cumulative administration of isoprenaline. The inotropic responses to the agonists were expressed as percentages 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 to wash out the blood. The experimental procedure was the same as that described previously (Takanashi and Endoh, 1991). Slices of ventricular muscle (0.5 mm thick) were prepared with a tissue slicer (Arthur H. Thomas Company, Philadelphia, PA) in cold (4°C) Krebs-Henseleit solution. After weighing, 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 fresh solution that contained 5 mM *myo*-inositol and 10 mM LiCl. All succeeding procedures were performed in Li^+-containing solution. (±)-Bupranolol (0.3 μM) was added to the solution 20 min prior to administration of the agonist to avoid interference by activation of β-adrenoceptors. The protocol employed for examination of the combined action of methoxamine and endothelin isopeptides on the accumulation of [^{3}H]inositol monophosphate (IP_1), which was used as an indicator of the acceleration of the hydrolysis of phosphoinositide, was the same as that described for the functional study. 30

min after the administration of an endothelin isopeptide, slices were quickly blotted and put into 1 ml of a mixture of chloroform, methanol and 12 N HCl (100:200:1, v/v) to terminate the reaction. The tissue was homogenized and then the homogenate was 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). [^{3}H]IP_1 was collected and the radioactivity was quantitated 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%.

2.3. Binding of [^{125}I]endothelin-3

The binding assay with [^{125}I]endothelin-3 was carried out as described in detail elsewhere (Takanashi and Endoh, 1991). In brief, pieces of right and left ventricular muscle, including free walls and septum, were excised from the rabbit heart and homogenized in ten volumes of ice-cold buffer (0.25 M sucrose containing 5 mM Tris-HCl and 1 mM $MgCl_2$, pH 7.4) in a Polytron PT-10 (Kinematica, Luzern, Switzerland) three times for 15 s at setting 7. The homogenate was then centrifuged at 500 × *g* for 15 min at 4°C. The supernatant was filtered through a single layer of cheesecloth and centrifuged at 50 000 × *g* for 20 min at 4°C. The resulting pellet was washed twice with ice-cold incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% bovine serum albumin, pH 7.5) by repeated resuspension and recentrifugation. The final pellet was resuspended in ice-cold incubation buffer at a protein concentration of about 1 mg/ml.

The specific binding assay was carried out in an incubation mixture that contained 150 μl of a suspension of membranes (approximately 100–150 μg of protein) and 50 μl of a solution of [^{125}I]endothelin-3 in a final volume of 250 μl for 90 min at 25°C in the presence or absence of competing agents at various concentrations. The incubation was terminated by adding 2 ml of ice-cold incubation buffer and the mixture was rapidly filtered through a GF/C glass filter (Whatman International, Maidstone, England) in an M-24R type cell harvester (Brandel, Gaithersburg, Maryland, USA). Each filter was rapidly washed with three 4-ml aliquots of ice-cold incubation buffer. After drying the filter for 1 h at 90°C, radioactivity bound to the filter was quantitated. Saturation curves were obtained by incubation of the cell membranes with increasing concentrations of [^{125}I]endothelin-3 (0.5–250 pM) and data were analyzed by construction of Scatchard plots. To investigate the influence of methoxamine on the maximum binding of [^{125}I]endothelin-3, the suspension of membranes was incubated with 0.1 mM methoxamine or with incubation buffer for 30 min at 37°C, and then the membranes were used in the binding assay with [^{125}I]endothelin-3. Nonspecific binding of [^{125}I]endothelin-3 was examined in the presence of 0.1 μM endothelin-3 and specific binding was defined as total radioactivity minus radioactivity due to nonspecific binding. Protein was quantitated by the method of Lowry et al. (1951).

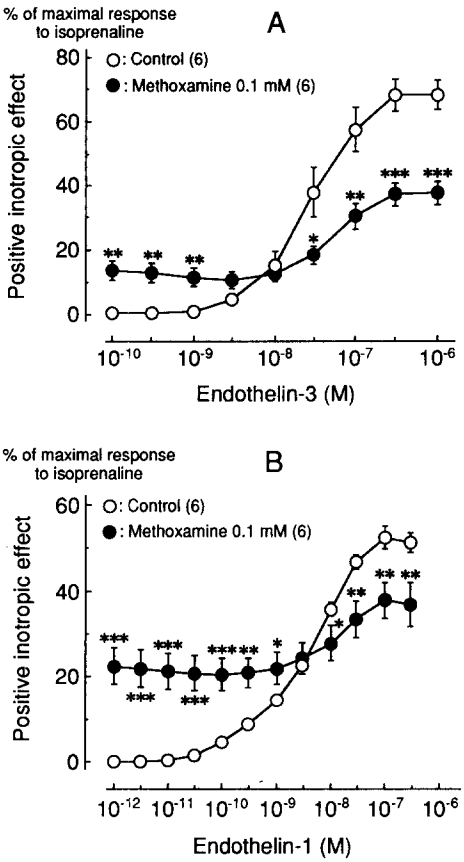

Fig. 1. Cumulative concentration-response curves for endothelin-3 (A) and endothelin-1 (B) in the absence (○) and presence of (●) methoxamine (0.1 mM), but always in the presence of 0.3 μM bupranolol, in isolated rabbit papillary muscle (0.5 Hz, 37°C). Methoxamine was allowed to pretreatment for 30 min and the concentration-response curves for endothelins were determined in the presence of methoxamine. Numbers in parentheses indicate numbers of experiments. The dimensions of the papillary muscles used in (A) and (B) were as follows: length 5.07 ± 0.40 and 5.07 ± 0.31 mm, cross-sectional area 0.64 ± 0.06 and 0.67 ± 0.07 mm^2, respectively (n = 12, each). The basal force of contraction and the maximum force in response to isoprenaline were 2.61 ± 0.45 and 27.1 ± 6.7 mN/mm^2 in (A), and 5.53 ± 1.04 and 33.2 ± 6.0 mN/mm^2 in (B), respectively. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. the corresponding control values.

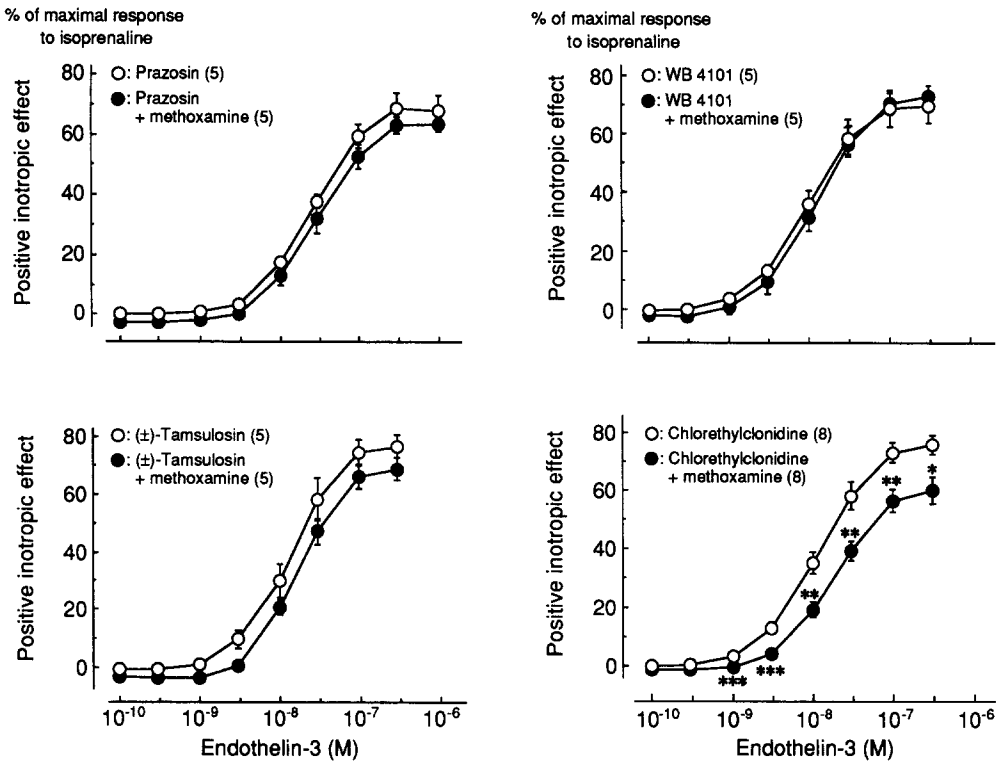

Fig. 2. Influence of 1 μM prazosin, 0.1 μM WB 4101, 0.1 μM (±)-tamsulosin, and 10 μM chlorethylclonidine on the inhibitory action of methoxamine on the positive inotropic effect of endothelin-3 in the presence of 0.3 μM bupranolol in isolated rabbit papillary muscle (0.5 Hz, 37°C). The dimensions of the papillary muscles used in the absence and presence of methoxamine were as follows: length 5.23 ± 0.22 and 5.44 ± 0.21 mm, cross-sectional area 0.49 ± 0.04 and 0.52 ± 0.04 mm^2, respectively ($n = 23$ each). The basal force of contraction and the maximum force in response to isoprenaline were 4.42 ± 0.78 and 41.0 ± 4.3 mN/mm^2, respectively (in experiments without methoxamine), and 5.06 ± 0.76 and 41.1 ± 4.5 mN/mm^2, respectively (in experiments with methoxamine). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. the corresponding control values.

2.4. Statistical analysis

Experimental values are presented as means ± S.E.M. Significant differences between mean values were estimated by means of one-way analysis of variance and/or by Student's *t*-test. A *P* value smaller than 0.05 was considered to be significant.

2.5. Drugs and radiolabeled compounds

The drugs and other agents used were methoxamine hydrochloride, (−)-isoprenaline hydrochloride, *myo*-inositol, lithium chloride, dihydroouabain, and bovine serum albumin (Sigma, St. Louis, MO, USA); Bay k 8644 (Bayter, Wuppertal, FRG); forskolin (Nippon

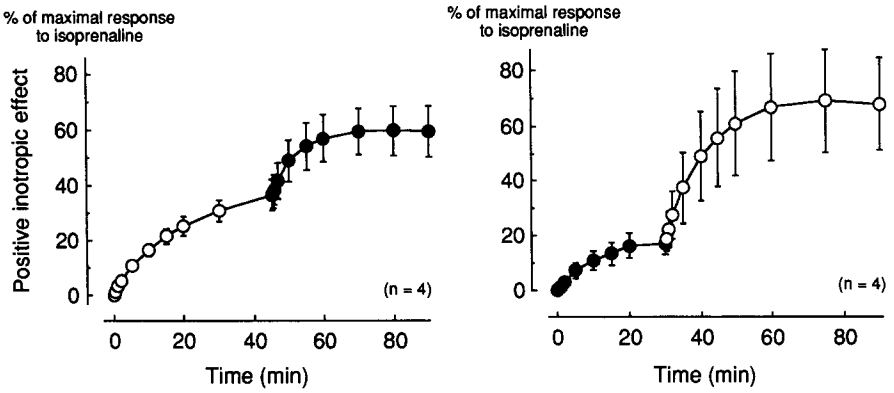

Fig. 3. Influence of 0.1 mM methoxamine (●) on the positive inotropic effect of 1 μM Bay k 8644 (○) in the presence of 0.3 μM bupranolol in isolated rabbit papillary muscle (0.5 Hz, 37°C). The dimensions of the papillary muscles used were as follows: length 4.50 ± 0.23 mm and cross-sectional area 0.55 ± 0.04 mm^2, respectively ($n = 8$). The basal force of contraction and the maximum force in response to isoprenaline were 4.85 ± 2.09 and 23.8 ± 6.2 mN/mm^2, respectively.

Kayaku Co., Tokyo, Japan); endothelin-1 and endothelin-3 (Peptide Institute, Osaka, Japan); prazosin hydrochloride (Pfizer Taito, Tokyo, Japan); (±)-tamsulosin (5-[2-[[2-(*o*-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide) hydrochloride (Yamanouchi Pharmaceutical Co., Tokyo, Japan); WB 4101 and chlorethylclonidine dihydrochloride (Research Biochemicals, Natick, MA, USA); ammonium formate (Wako Pure Chemicals Co., Osaka, Japan); (±)-bupranolol hydrochloride (Kaken Pharmaceutical Co., Tokyo, Japan); pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA); and [^{125}I]endothelin-3 (specific activity, 2000 Ci/mmol) and *myo*-[2-^{3}H]inositol (specific activity, 86 Ci/mmol; Amersham, Buckinghamshire, UK). Before use, a stock solution of isoprenaline was freshly prepared in 0.1% ascorbic acid, kept on ice, and diluted with 0.9% NaCl as needed.

3. Results

3.1. Inhibition by methoxamine of the positive inotropic effects of endothelin isopeptides

Methoxamine at 0.1 mM increased the force of contraction by 19.1 ± 2.4% of the maximal response to isoprenaline ($n = 12$). In the presence of methoxamine, the positive inotropic effects of endothelin-3 and endothelin-1 were markedly suppressed (Fig. 1). In the presence of methoxamine, the maximal response to endothelin-3 was decreased by 44.8 ± 5.4% and the EC_{50} value for endothelin-3 was increased to 72.4 ± 13.1 nM from the control value of 33.5 ± 7.3 nM (Fig. 1A). The maximal response to endothelin-1 was decreased by 27.4 ± 7.0% and the EC_{50} value for endothelin-1 was increased from 4.3 ± 0.8 nM in the control to 20.4 ± 6.1 nM in the presence of methoxamine (Fig. 1B).

In next series of experiments, methoxamine was allowed to act for 15 min or 60 min prior to determination of concentration-response curves for endothelin-3 to investigate the influence of incubation time on the methoxamine-induced inhibition. The positive inotropic effect of methoxamine reached the peak at 30 min and remained unchanged up to 60 min. The extent of inhibition induced by methoxamine after preincubation for 15 min (29.2 ± 4.6%, $n = 5$) was not significantly ($P > 0.05$) different from that achieved after preincubation with methoxamine for 60 min (35.3 ± 4.6%, $n = 5$), indicating that the inhibitory action of methoxamine might have been fully achieved after preincubation for 30 min.

In another series of experiments, it was investigated whether other sympathomimetic amines, noradrenaline and phenylephrine, mimic the inhibitory action of methoxamine. When 3 μM noradrenaline or 1 μM phenylephrine was allowed to act for 30 min and the cumulative concentration-response curves for endothelin-3 were determined in their presence, the positive inotropic effect of endothelin-3 was attenuated by 12.9 ± 2.1% ($n = 5$, $P < 0.05$) or by 20.6 ± 7.0% of the control ($n = 2$), respectively, while noradrenaline or phenylephrine alone increased the force of contraction by 20.2 ± 2.6% and 16.9 ± 3.6% of the maximal response to isoprenaline, respectively. The EC_{50} value for endothelin-3 was increased from 5.6 ± 1.0 nM (control) to 10.4 ± 2.7 nM ($P < 0.05$) in the presence of noradrenaline, and from 10 ± 0.7 nM (control) to 18.2 ± 1.7 nM in the presence of phenylephrine.

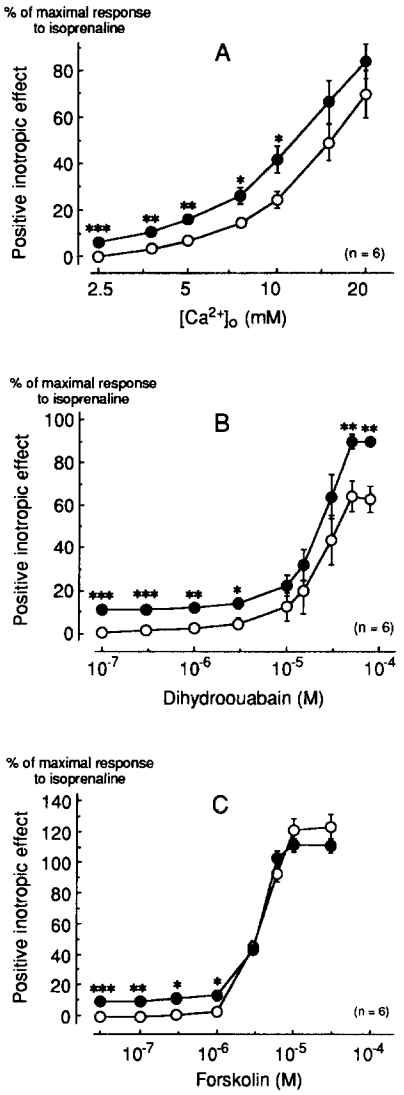

Fig. 4. Cumulative concentration-response curves for the elevation of the level of extracellular Ca^{2+} ions (A), for dihydroouabain (B) and for forskolin (C) in the absence (○) and presence of (●) methoxamine (0.1 mM) in the presence of 0.3 μM bupranolol, in isolated rabbit papillary muscle (0.5 Hz, 37°C). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. the corresponding control values.

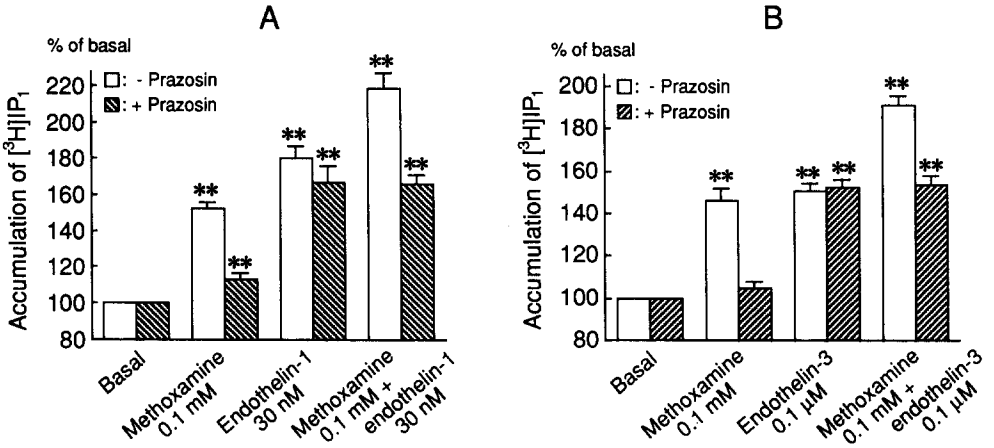

Fig. 5. Influence of methoxamine on the accumulation of [^{3}H]inositol monophosphate ([^{3}H]IP_1) induced by endothelin-1 (A) or by endothelin-3 (B) in the absence or presence of 0.3 μM (A) or 1 μM (B) prazosin, but always in the presence of 0.3 μM bupranolol, in rabbit ventricular slices. Data are given as percentages of the corresponding basal control levels that were determined simultaneously. The mean radioactivity of control slices was 20.7 ± 4.0 dpm/mg wet weight in A (n = 4) and 27.8 ± 4.3 dpm/mg wet weight in B (n = 6). $^{**}P < 0.01$ vs. the corresponding control values.

3.2. Influence of α_1-adrenoceptor antagonists on inhibitory action of methoxamine

Prazosin (1 μM), WB 4101 (0.1 μM) and (±)-tamsulosin (0.1 μM) did not affect the baseline force of contraction but they abolished the positive inotropic effect of methoxamine, as well as the inhibitory action of methoxamine on the positive inotropic effect of endothelin. Thus, the concentration-response curves for the positive inotropic effect of endothelin-3 in the presence of methoxamine and α_1-adrenoceptor antagonist, prazosin, or α_{1A}-adrenoceptor antagonists, WB 4101 and (±)-tamsulosin, together were superimposable on the curves for endothelin-3 alone (Fig. 2).

Pretreatment with chlorethylclonidine (10 μM) also abolished the positive inotropic effect of methoxamine (0.1 mM), while chlorethylclonidine reduced the inhibitory action of methoxamine to a lesser extent (Fig. 2). In muscle that had been pretreated with chlorethylclonidine, methoxamine inhibited the maximal inotropic response to endothelin-3 by 15.7 ± 4.6%, the EC_{50} value for endothelin-3 increased from the control value of 12.4 ± 1.9 nM to 18.7 ± 2.2 nM in the presence of methoxamine ($P < 0.05$).

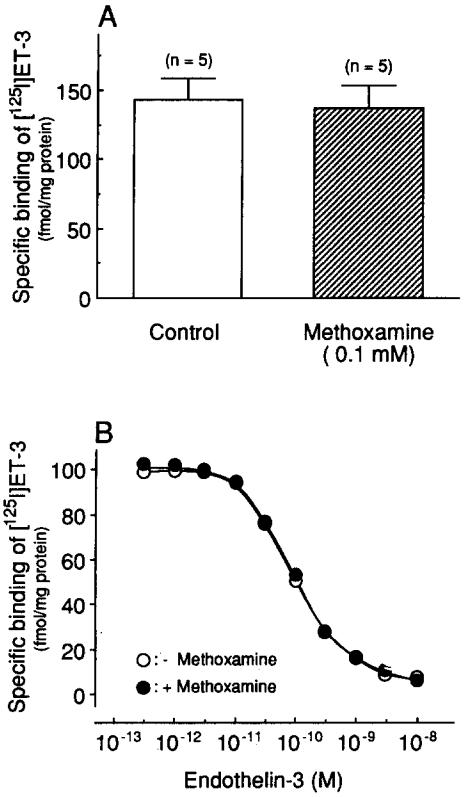

Fig. 6. Influence of pretreatment with 0.1 mM methoxamine on the specific binding of [^{125}I]endothelin-3 at the saturating concentration of 0.2 nM (A) and on the endothelin-3-induced displacement of the special binding of [^{125}I]endothelin-3 (B) in membranes derived from rabbit ventricular muscle (n = 5, each).

3.3. Influence of methoxamine on positive inotropic effects that are not associated with the hydrolysis of phosphoinositide

Fig. 3 shows the effects of methoxamine applied with Bay k 8644. The positive inotropic effect of Bay k 8644 was unaffected by methoxamine. The positive inotropic effects of these agents were additive in respective of whether methoxamine was administered during induction of the effect of Bay k 8644 or prior to addition of Bay k 8644 (Fig. 3).

The concentration-response curves for the positive inotropic effects of the elevation of $[Ca^{2+}]_o$ (Fig. 4A), of dihydroouabain (Fig. 4B) and of forskolin (Fig. 4C) were unaffected by methoxamine: the effects of the first two interventions were additive with respect to

that of methoxamine, while the effect of forskolin was reduced slightly but not significantly.

3.4. Interactive effects of methoxamine and endothelin isopeptides on the accumulation of [^{3}H]IP$_1$

In the previous study, we have shown that the accumulation of [^{3}H]IP$_1$ is an excellent indicator of the receptor-mediated acceleration of the hydrolysis of phosphoinositide (Yang and Endoh, 1994). Therefore, we examined the effect of methoxamine on the accumulation of [^{3}H]IP$_1$ induced by endothelin isopeptides. The accumulation of [^{3}H]IP$_1$ that was induced by endothelin-1 (Fig. 5A) or by endothelin-3 (Fig. 5B) was not inhibited by methoxamine and the extent of the accumulation induced by either isopeptide and methoxamine was additive. Prazosin abolished the accumulation of [^{3}H]IP$_1$ induced by methoxamine but it did not affect the accumulation caused by the isopeptides (Fig. 5).

3.5. Influence of methoxamine on the specific binding of [^{125}I]endothelin-3

We further examined whether methoxamine affects the specific binding of endothelin-3 to the membrane of the rabbit ventricular muscle. Methoxamine affected neither the B_{max} (the maximal binding capacity) of the specific binding of [^{125}I]endothelin-3 (Fig. 6A) nor did it affect the curve that demonstrated the ability of endothelin-3 to displace [^{125}I]endothelin-3 from a membrane fraction derived from rabbit ventricular muscle (Fig. 6B).

4. Discussion

Methoxamine alone had a positive inotropic effect on isolated rabbit papillary muscle. This effect was abolished by prazosin, WB 4101, (±)-tamsulosin and chlorethylclonidine, an indication that the effect was mediated by α_1-adrenoceptors, as shown in previous studies (Endoh and Schümann, 1975; Yang and Endoh, 1994). In addition, methoxamine had a pronounced inhibitory action on the positive inotropic effects of endothelin-1 and endothelin-3 in rabbit papillary muscle. The maximal inotropic responses to endothelin isopeptides were significantly decreased and the EC_{50} values were increased. In this respect, the methoxamine-induced inhibitory action was similar to that on the α_1-adrenoceptor-mediated positive inotropic effect (Yang and Endoh, 1994). Several important characteristics of the methoxamine-induced inhibitory effect were revealed in the present study as follows.

Although it has been reported that methoxamine has a nonspecific, direct, depressant effect on myocardial contractility and cardiac membrane ion channels (for review, see Endoh, 1982), the inhibitory action of methoxamine on the endothelin-induced positive inotropic effect observed in the present study is not likely to have been due to such a nonspecific effect because the positive inotropic effects induced by elevation of $[Ca^{2+}]_o$, by Bay k 8644, by dihydroouabain and by forskolin were not significantly affected by methoxamine.

Furthermore, the inhibitory action of methoxamine was abolished by an α_1-adrenoceptor antagonist, prazosin and selective α_{1A}-adrenoceptor antagonists, WB 4101 (Han et al., 1987; Minneman, 1988) and (±)-tamsulosin (Hanft et al., 1989; García-Sáinz et al., 1995); and it was partially attenuated by an α_{1B}-adrenoceptor antagonist, chlorethylclonidine (Han et al., 1987; Minneman, 1988). These results indicate that the inhibitory action of methoxamine is mediated by α_1-adrenoceptors or, more specifically, by α_{1A}-adrenoceptors.

Methoxamine inhibited the positive inotropic effects of phenylephrine (Yang and Endoh, 1994) and of endothelin isopeptides, both of which have a positive inotropic effect in association with the acceleration of the hydrolysis of phosphoinositide (Otani et al., 1988; Takanashi et al., 1991; Takanashi and Endoh, 1991). Therefore, it is postulated that the inhibitory action of methoxamine might be selectively exerted on the signal-transduction process that is related to the hydrolysis of phosphoinositide. In this context, it is noteworthy that methoxamine affected neither the binding characteristics of [^{125}I]endothelin-3 nor the accumulation of [^{3}H]IP$_1$ that was induced by endothelin isopeptides. These results imply that methoxamine might act subsequent to the acceleration of phosphoinositide hydrolysis.

As concerned with the mechanism underlying the inhibitory action of methoxamine, it has recently been reported that the activation of protein kinase C is coupled to phosphorylation of contractile proteins to lead to inhibition of the positive inotropic effect (Strang and Moss, 1995). Considering the current findings that there are multiple isoenzymes of protein kinase C with different catalytic properties in cardiac muscle (for review, see Steinberg et al., 1995), we postulate that the coupling processes subsequent to activation of different types of receptor that induce an acceleration of phosphoinositide hydrolysis might be segregated and result in activation of different isoenzymes of protein kinase C. This postulate is supported by the observation that the phorbol ester (phorbol 12,13-dibutyrate) produced an action that may be completely different from endogenously generated diacylglycerol in the rabbit-ventricular myocardium. Phorbol 12,13-dibutyrate inhibited the acceleration of phosphoinositide hydrolysis and the positive inotropic effect induced by α-

adrenoceptor agonists, endothelin or angiotensin II, while these receptor agonists showed no tachyphylaxis (α-adrenoceptor agonists) or only a small extent of tachyphylaxis by repetition (angiotensin II) (Endoh et al., 1993; Endoh and Takanashi, 1991; Ishihata and Endoh, 1993). Finally, we showed that the inhibitory action of methoxamine is mediated by specific subtypes of adrenoceptors. This adrenoceptor subtype may have physiological relevance to contractile regulation in the rabbit ventricular myocardium, because other sympathomimetic amines such as noradrenaline and phenylephrine mimicked the inhibitory action of methoxamine.

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