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Effects of long-term pretreatment with isoproterenol on inotropic responsiveness to α-adrenoceptor stimulation: study in isolated perfused rat hearts

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Abstract

The effects of chronic pretreatment with isoproterenol (5 mg kg^{-1}) daily for 10 days on cardiac α-adrenergic responsiveness in Langendorff heart preparations were investigated. Isoproterenol pretreatment caused cardiac hypertrophy (29%) as shown by a significant increase in the ratio of ventricular dry weight to body weight. In preparations from isoproterenol-pretreated rats, both maximum increases in left ventricular systolic pressure and heart rate elicited by isoproterenol (10^{-12} to 10^{-4} M) were significantly reduced (the isoproterenol concentration producing 50% of the maximum positive inotropic and chronotropic responses was enhanced almost 32- and 4-fold, respectively), while the positive inotropic response to phenylephrine (10^{-12} to 10^{-4} M) was significantly enhanced (the phenylephrine concentration producing 50% of the maximum positive inotropic effect was reduced almost 100-fold), compared with saline-pretreated rats. In preparations from both groups, phenylephrine infusion induced non-significant changes in heart rate and its positive inotropic response was reduced in the presence of propranolol (10^{-7} M) in the perfusion medium. Even under β-adrenoceptor blockade, the curve for the phenylephrine-induced positive inotropic effect remained shifted upward after isoproterenol pretreatment. Chronic isoproterenol pretreatment induces the expected cardiac β-adrenoceptor desensitization while simultaneously enhancing the positive inotropic responsiveness to phenylephrine in Langendorff heart preparations. These findings support the hypothesis that cardiac α_1-adrenoceptor stimulation may contribute to the maintenance of myocardial function under conditions in which β-adrenoceptor function is compromised.

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Introduction

It is well known that β-adrenoceptors are the predominant class of adrenoceptor in the heart, mediating both positive inotropic and chronotropic responses. However, the existence of functional myocardial α_1-adrenoceptors in different cardiac preparations from several species, including man, has been well characterized in both biochemical and functional studies (Schumann et al 1978; Wagner & Brodde 1979; Brückner et al 1984, 1985; Aass et al 1986; Terzic et al 1993). In contrast to the effects mediated by myocardial β_1-adrenoceptor stimulation, activation of post-junctional α_1-adrenoceptor in the myocardium increases contractile force with little or no change in cardiac rate (Benfey 1982). While the positive inotropic response to

cardiac β-adrenoceptor activation is related to increased levels of cyclic AMP, cardiac α_1-adrenoceptor stimulation transduces signals principally via changes in intracellular free calcium (Minneman 1988; Scholz 1989; Terzic et al 1993). The mode of action by which cardiac α_1-adrenoceptor stimulation induces a positive inotropic effect is not yet completely elucidated. Several mechanisms have been proposed, including: an indirect increased influx of extracellular calcium leading to prolongation of the action potential; degradation of polyphosphoinositides, particularly phosphatidylinositol 4,5-biphosphate (PIP_2), leading to inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG) formation. IP_3 induces calcium influx, which together with DAG, activates protein kinase C (Minneman 1988; Scholz 1989; Kaku et al 1991); and an increase in myofibrillar responsiveness to calcium (Terzic et al 1993). The relative contribution of these proposed mechanisms is still under investigation.

The precise role of α_1-adrenoceptor stimulation in myocardial contractility is not yet clear. Under normal conditions, β_1-adrenoceptor-mediated responses predominate over those elicited by α_1-adrenoceptors. However, changes in the number of α_1-adrenoceptors have been reported under certain experimental and pathological conditions. For instance, whereas this parameter remained unchanged in hypertensive animals (Limas & Limas 1987; Mertens et al 1992), the number of myocardial α_1-adrenoceptors was reduced in experimentally-induced diabetes mellitus (Tanaka et al 1992). However, α_1-adrenoceptor density in myocardium increased after chronic treatment of rats with the β-adrenoceptor antagonist, propranolol (Mügge et al 1985; Steinkraus et al 1989), a condition where inotropic responsiveness to β_1-adrenoceptor activation is reduced (Mügge et al 1985). Using pretreatment with isoproterenol (ISO; 40 μg kg^{-1} daily for 3 days), a well-known model of compensatory hypertrophy associated with cardiac β-adrenoceptor desensitization, Butterfield & Chess-Williams (1993) showed that the responsiveness to α_1-adrenoceptor stimulation is enhanced in rat papillary muscle preparations. Thus, it has been speculated that myocardial α_1-adrenoceptor stimulation may contribute to the maintenance of myocardial performance in situations in which cardiac β-adrenoceptor function is impaired (Brückner et al 1985; Osnes et al 1985; Homcy et al 1991; Butterfield & Chess-Williams 1993). The aim of this study was to further corroborate this latter hypothesis. Therefore, we investigated the effects of long-term pretreatment with ISO on inotropic responsiveness to α-adrenoceptor stimulation in isolated, perfused Langendorff rat hearts.

Materials and Methods

Drugs

(−)-Isoproterenol hydrochloride, (−)-phenylephrine hydrochloride (PHE) and DL-propranolol hydrochloride (PROP) were purchased from Sigma Chemical Co. (St Louis, MO) and were dissolved in saline just before use. Solutions were made fresh each day and kept in the dark. Heparin (Laboratoires Léo S.A., Montigny-le-Bretonneux, France) was used as the commercially available injectable solution.

Isoproterenol pretreatment

Male Wistar rats (250–270 g) were kept under conditions of constant temperature (23 ± 2°C) with a standard 12-h light–dark cycle, and had free access to food and water. In each series of experiments, the rats were divided randomly into two groups. The ISO group was pretreated daily with intraperitoneal injections of ISO (5 mg kg^{-1} in saline) (Verdetti & Mezin 1980) for 10 days. The control group received saline (1 mL kg^{-1}, i.p.) and was treated in the same way. The bodyweight of all rats was determined every second day. Functional in-vitro experiments were performed 24 h after the last treatment with ISO or saline.

Perfusion model

At the time of experiments, rats were stunned, intravenously heparinized (1000 IU kg^{-1}) and killed by cervical dislocation. After thoracotomy, hearts were quickly excised and arrested in ice-cold perfusion medium. After removal of lung and fat tissue, hearts were immediately mounted and perfused according to the Langendorff technique at a constant perfusion pressure of 80 mmHg. Retrograde perfusion of the aorta was achieved with filtered (Millipore; 0.65 μm), modified Krebs-Henseleit solution (pH = 7.4) containing (mM): NaCl 129, KCl 5.6, $MgCl_2$ 1.25, $NaHCO_3$ 21, $CaCl_2$ 1.25, NaH_2PO_4 1.2. Glucose (10 mM) was added as a substrate. Sodium pyruvate (2 mM) was added as a cosubstrate to preserve myocardial performance during the 120-min perfusion period (Van Bilsen et al 1988). This buffer was continuously aerated with 5% CO_2 in oxygen and maintained at 37°C. Under these conditions the hearts beat spontaneously.

Recording of cardiac parameters

Left ventricular systolic pressure (LVSP) was measured through a cannulated, water-filled latex balloon inserted

through the atrium into the left ventricle, and connected by a polyethylene catheter to a blood pressure transducer, which in turn was connected to a Gilson model 5/6 H polygraph (Gilson Medical Electronics Inc., Middletown, WI). Heart rate (HR) was calculated from the LVSP tracing. The collapsed balloon attached to the transducer was secured in place and filled with water to a volume that produced a left ventricular diastolic pressure (LVDP) of approximately 10 mmHg.

Experimental protocol

After perfusing the hearts for an equilibration period of 30 min, baseline measurements of LVSP, LVDP and HR were made, and the following experiments were performed in preparations from both saline- and ISO-pretreated rats. In the first series of experiments, a concentration–effect curve was made with increasing bolus (0.1 mL) concentrations of ISO, over the range of 10^{-12} to 10^{-4} M. ISO boluses were separated by 5–10 min intervals, allowing LVSP and HR values to return to their respective baseline levels, as established during the equilibration period. In the second series of experiments, maximum percentage changes in LVSP and HR to bolus (0.1 mL) concentrations of PHE (10^{-12} to 10^{-4} M) were determined. Only one concentration of PHE could be achieved in each preparation. The third series of experiments was performed to examine the effects of cardiac β-adrenoceptor blockade with PROP on PHE-induced changes in LVSP and HR. Therefore, after the 30-min equilibration period, baseline LVSP and HR were measured and hearts were further perfused with a solution of PROP (10^{-7} M) prepared in perfusion medium. After another 30-min equilibration period, baseline LVSP and HR were again measured, and then maximum percentage changes in LVSP and HR elicited by bolus concentrations of PHE (10^{-12} to 10^{-4} M) were determined. In all experiments, maximum changes in LVSP and HR evoked by ISO or PHE in both saline- and ISO-pretreated rats occurred within 15 s after drug infusion.

Determination of cardiac hypertrophy

Cardiac hypertrophy was determined in another set of saline- or ISO-pretreated rats. Rats were killed by cervical dislocation and the hearts excised. Large blood vessels and atria were removed and the ventricles were rinsed with saline and blotted with filter paper. They were weighed, dried for 48 h in a desiccator at 70°C and reweighed (Knufman et al 1987). The ratio of dry ventricular weight to bodyweight was used as an index of cardiac hypertrophy.

Determination of pD_2

Concentration–effect curves were obtained by plotting the maximum increases in LVSP and HR (expressed as percentage of baseline values) as a function of the negative logarithm of drug concentration. The pD_2 value was defined as the negative logarithm of agonist concentration (M) producing 50% of the maximum effect (EC50). In experiments with ISO, this value was determined graphically in each individual concentration–effect curve. The geometric mean pD_2 was calculated by averaging the pD_2 values of each concentration–effect curve. In experiments with PHE, since only one concentration of this agonist was tested in each preparation, only the apparent geometric pD_2 was determined from the mean concentration–effect curve.

Statistical analysis

All the results are expressed as means ± s.e.m. Changes in absolute LVSP and HR with respect to individual baseline values were computed. One-way analysis of variance (groups) and unpaired or paired Student's *t*-tests were used to assess significant differences in baseline LVSP and HR between the different groups. Statistical significance of LVSP and HR responses to ISO and to PHE alone, or in association with PROP, was determined using the paired Student's *t*-test. Comparisons between saline- and ISO-pretreated groups were performed using the unpaired Student's *t*-test and Mann-Whitney U-test. Groups of data were compared with one-way (concentrations) or two-way (treatment × concentrations) analysis of variance tests. Differences were considered statistically significant at $P < 0.05$.

Results

Effects of ISO pretreatment on bodyweight and heart weight

Pretreatment with ISO did not affect bodyweight, but significantly increased ($P < 0.001$, unpaired Student's *t*-test) both ventricular wet and dry weights (Table 1). Such treatment resulted in myocardial hypertrophy (29%) as shown by the significant increase ($P < 0.001$, unpaired Student's *t*-test) in the ratio of ventricular dry weight to final bodyweight (Table 1).

Table 1 Effects of 10-day pretreatment with isoproterenol (5 mg kg^{-1}, i.p daily) on heart and bodyweight.

	Saline-pretreated rats	ISO-pretreated rats
Initial bodyweight (g)	260 ± 2	264 ± 4
Final bodyweight (g)	296 ± 4‡	289 ± 3‡
Ventricular wet weight (mg)	948 ± 32	1040 ± 5*
Ventricular dry weight (mg)	187 ± 7	234 ± 6**
Ventricular dry weight/bodyweight (mg g^{-1})	0.63 ± 0.02	0.81 ± 0.02**

Values are means $\pm$ s.e.m. (n = 13 rats per group). ‡$P < 0.001$ vs initial bodyweight of saline- or ISO-pretreated rats (paired Student's *t*-test). *$P < 0.05$, **$P < 0.001$ vs saline-pretreated rats (unpaired Student's *t*-test).

Effects of ISO pretreatment on baseline LVSP and HR and their changes in response to increasing concentrations of ISO (Series 1)

In this series of experiments, baseline LVDP values (average 10 mmHg) in Langendorff preparations from ISO-pretreated rats were of the same order of magnitude as those recorded in control preparations (i.e. from saline-pretreated rats), and remained stable throughout the entire recording period (data not shown). Baseline values of LVSP, however, were significantly higher in ISO-pretreated (92 ± 2 mmHg, n = 6) than in saline-pretreated (71 ± 2 mmHg, n = 6) rat hearts ($P < 0.001$, unpaired Student's *t*-test). In contrast, baseline HR values in ISO-pretreated rats (223 ± 4 beats min^{-1}) were of the same order of magnitude ($P > 0.05$, unpaired Student's *t*-test), as that of saline-pretreated rats (228 ± 4 beats min^{-1}). In both groups, baseline values of LVSP and HR remained stable throughout the experimental period ($P > 0.05$, one-way analysis of variance).

In preparations from saline-pretreated rats, ISO infusion evoked the expected concentration-dependent increase in LVSP and HR (Figure 1; $P < 0.001$, one-way analysis of variance). Both effects were significant at concentrations of 10^{-12} and 10^{-10} M, respectively ($P < 0.05$, paired Student's *t*-test with respect to baseline values). In hearts from ISO-pretreated rats, the same treatment also increased LVSP and HR in a concentration-dependent manner (Figure 1; $P < 0.001$, one-way analysis of variance). These responses were also significant at concentrations of 10^{-12} and 10^{-10} M, respectively ($P < 0.05$, paired Student's *t*-test). They were significantly diminished over the whole concentration range used (except at 10^{-12} M), when compared with control rats (Figure 1; $P < 0.05$, Mann-Whitney U-test). The curves for both ISO-induced maximum percentage increase in LVSP and HR were shifted downward after 10-day ISO pretreatment (Figure 1; $P < 0.001$, two-way analysis of variance). In saline- and ISO-pretreated hearts, the pD_2 values were 8.38 ± 0.22 and 6.88 ± 0.31 for LVSP, and 8.86 ± 0.22 and 8.24 ± 0.27 for HR, respectively, yielding ratios of EC50 ISO to EC50 saline of 31.6 and 4.2 for LVSP and HR, respectively.

Effects of ISO pretreatment on LVSP and HR responses to PHE at increasing concentrations (Series 2)

In this series of experiments, baseline LVDP values in preparations from ISO-pretreated rats were also of the same order of magnitude as those recorded in control preparations, and also remained stable throughout the entire recording period (data not shown). As was shown in Series 1, baseline values of LVSP recorded in this series of experiments were significantly higher in ISO-pretreated (86 ± 3 mmHg, n = 8) than in saline-pretreated (72 ± 1 mmHg, n = 6) rat hearts ($P < 0.001$, unpaired Student's *t*-test). However, baseline values of HR were of the same order of magnitude, irrespective of whether the rat was or was not chronically pretreated with ISO (241 ± 5 and 228 ± 5 beats min^{-1}, respectively). In both groups, baseline values of LVSP and HR remained stable throughout the recording period ($P > 0.05$, one-way analysis of variance). Finally, neither baseline parameter varied significantly from those in Series 1 ($P > 0.05$, unpaired Student's *t*-test).

In preparations from both saline- and ISO-pretreated rats, PHE infusion induced a concentration-dependent increase in LVSP (Figure 2; $P < 0.001$, one-way analysis of variance), an effect which was significant at concentrations of 10^{-10} and 10^{-12} M, respectively ($P < 0.05$, paired Student's *t*-test). In preparations from ISO-

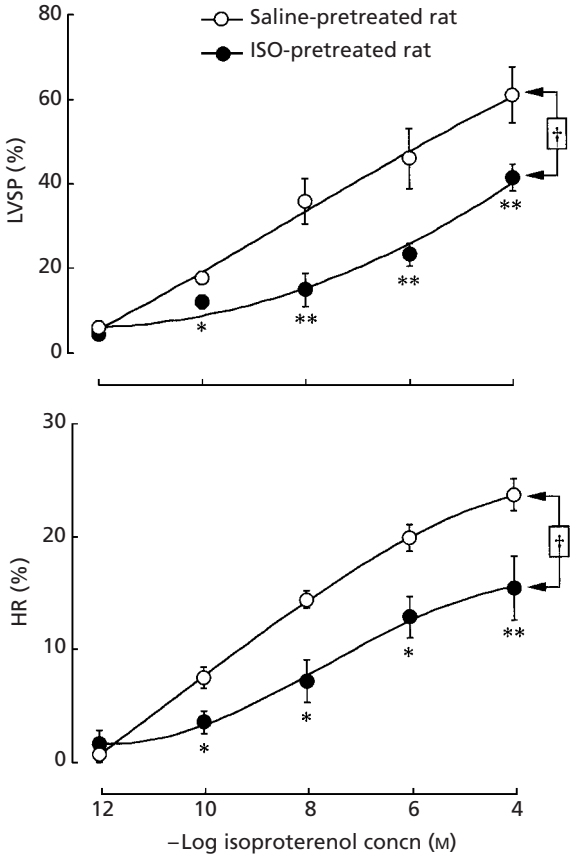

Figure 1 Maximum percentage increase in left ventricle systolic pressure (LVSP) and heart rate (HR) during infusion of increasing bolus concentrations of isoproterenol (ISO; 10^{-12} to 10^{-4} M) in isolated, perfused hearts from saline- and ISO-pretreated rats. Vertical bars indicate s.e.m. (n = 6 rats per group). The curves of the ISO-induced increase in LVSP and HR were shifted downward after 10-day ISO pretreatment (†$P < 0.001$, two-way analysis of variance). *$P < 0.05$, **$P < 0.01$ vs saline-pretreated rats (Mann-Whitney U-test).

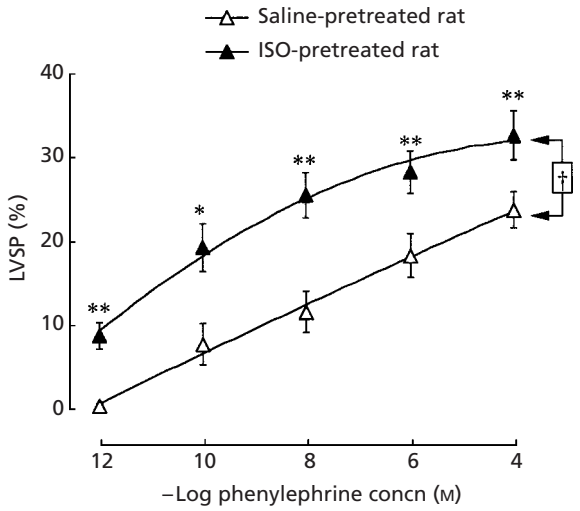

Figure 2 Maximum percentage increase in left ventricular systolic pressure (LVSP) during infusion of increasing bolus concentrations of phenylephrine (10^{-12} to 10^{-4} M) in isolated, perfused hearts from saline-pretreated and isoproterenol (ISO)-pretreated rats. Vertical bars indicate s.e.m. (n = 6–8 rats per group). The curve of the phenylephrine-induced percent increase in LVSP was shifted upward after 10-day ISO pretreatment (†$P < 0.001$, two-way analysis of variance). *$P < 0.05$, **$P < 0.01$ vs saline-pretreated rats (Mann-Whitney U-test).

pretreated rats, this effect was significantly enhanced over the whole concentration range used (Figure 2; $P < 0.01$, Mann-Whitney U-test with respect to control rats). The curve for the PHE-induced percentage increase in LVSP was shifted upward after 10-day ISO pretreatment (Figure 2; $P < 0.001$, two-way analysis of variance). Sensitivity to PHE, indicated by the apparent pD_2, was enhanced in ISO-pretreated hearts (10.10) compared with saline-pretreated (8.08) hearts. The difference in pD_2 values indicates an approximately 100-fold difference in sensitivity to PHE. In preparations from both groups, PHE infusion evoked non-significant ($P > 0.05$; paired Student's *t*-test) changes in HR over the whole concentration range used (data not shown).

Effects of propranolol on baseline LVSP and HR and their changes in response to increasing concentrations of PHE in saline- and ISO-pretreated rats (Series 3)

Before PROP (10^{-7} M) infusion, baseline LVDP values were also comparable in both saline- and ISO-pretreated rats, and remained stable throughout the experimental period (data not shown). Baseline values of LVSP, however, were significantly higher in ISO-pretreated (94 ± 4 mmHg, n = 10) than in saline-pretreated (69 ± 1 mmHg, n = 6) rat hearts ($P < 0.001$, unpaired Student's *t*-test). In contrast, baseline values of HR in ISO-pretreated rats (245 ± 8 beats min^{-1}) were of the same order of magnitude ($P > 0.05$, unpaired Student's *t*-test), as those of control rats (230 ± 6 beats min^{-1}). In both groups, baseline values of LVSP and HR remained stable throughout the experimental period ($P > 0.05$, one-way analysis of variance) and were comparable with those recorded in Series 2 ($P > 0.05$, unpaired Student's *t*-test). After a 30-min perfusion period with PROP, baseline LVSP and HR were significantly reduced ($P < 0.001$, paired Student's *t*-test). Maximum percentage decreases in baseline LVSP and HR evoked by PROP were of the same order of magnitude ($P >$

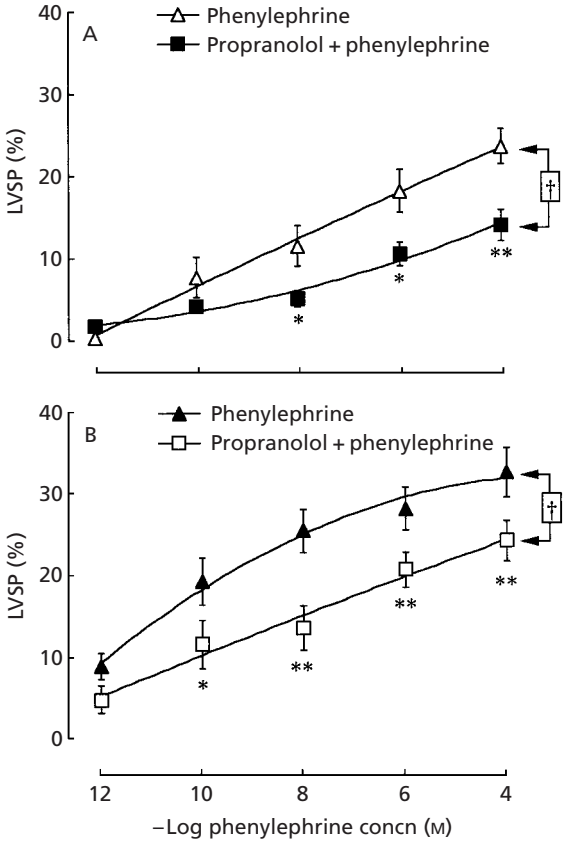

Figure 3 Effects of propranolol (10^{-7} M) on the maximum percentage increase in left ventricular systolic pressure (LVSP) during infusion of increasing bolus concentrations of phenylephrine (10^{-12} to 10^{-4} M) in isolated, perfused hearts from saline-pretreated (A) and isoproterenol-pretreated (B) rats. Vertical bars indicate s.e.m. (n = 6–10 rats per group). In both groups, the curve of the phenylephrine-induced percent increase in LVSP was shifted downward in the presence of PROP in the perfusion medium (†$P < 0.001$, two-way analysis of variance). *$P < 0.05$, **$P < 0.01$ vs saline-pretreated rats (Mann-Whitney U-test).

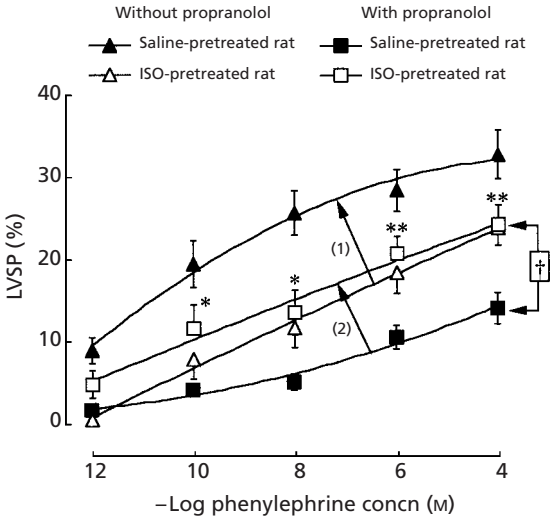

Figure 4 Effect of propranolol (10^{-7} M) on the enhanced maximum percentage increase in left ventricular systolic pressure (LVSP) induced by increasing bolus concentrations of phenylephrine (10^{-12} to 10^{-4} M) in isolated, perfused hearts from isoproterenol (ISO)-pretreated rats. Vertical bars indicate s.e.m. (n = 6–10 rats per group). Even under cardiac β-adrenoceptor blockade with propranolol, the curve of the phenylephrine-induced percentage increase in LVSP remained shifted upward after 10-day ISO pretreatment (†$P < 0.001$, two-way analysis of variance). Enhancement of phenylephrine-induced inotropic effect in the absence of propranolol (1); enhancement of phenylephrine-induced inotropic effect in the presence of propranolol (2). *$P < 0.05$, **$P < 0.01$ vs saline-pretreated rats (Mann-Whitney U-test).

0.05, Mann-Whitney U-test), irrespective of whether the rat was (25.4 ± 2.4 and 25.7 ± 3.3 %, respectively) or was not (26.2 ± 1.7 and 22.3 ± 2.8 %, respectively) chronically pretreated with ISO.

Under β-adrenoceptor blockade with PROP, infusion of either saline- (Figure 3A) or ISO-pretreated (Figure 3B) rat hearts with PHE increased LVSP in a concentration-dependent manner ($P < 0.001$, one-way analysis of variance), an effect which became significant at a concentration of 10^{-10} M ($P < 0.05$, paired Student's *t*-test). In both groups, the curve for the PHE-induced percentage increase in LVSP was shifted downward in the presence of PROP (Figure 3; $P < 0.001$, two-way analysis of variance). This attenuation became significant at concentrations of 10^{-8} and 10^{-10} M for saline- and ISO-pretreated rats, respectively (Figure 3; $P < 0.05$, Mann-Whitney U-test). Sensitivity to PHE was reduced by PROP, as indicated by the shift of the apparent pD_2 from 8.08 to 7.20 in saline-pretreated rats, and from 10.10 to 9.20 in ISO-pretreated rats. The new curves for both groups obtained under treatment with PROP are compared in Figure 4. A two-way analysis of variance showed that even under such conditions, the curve representing PHE-induced percentage increase in LVSP remained shifted ($P < 0.001$) upward after 10-day ISO pretreatment (Figure 4). This enhancement became significant at a concentration of 10^{-10} M (Figure 4; $P < 0.05$, Mann-Whitney U-test with respect to control rats). It is noteworthy that the difference in apparent pD_2 values for LVSP between ISO- and saline-pretreated rats (10.10 vs 8.08) was maintained in the presence of PROP (9.20 vs 7.20). A two-way analysis of variance revealed that in both groups, PHE-induced non-significant changes in HR were not statistically ($P > 0.05$) altered by PROP perfusion (data not shown).

Discussion

Pretreatment with ISO for 10 days induced no changes in rat bodyweight, but induced a significant increase in ventricular wet weight, which could have been due to development of cardiac oedema. However, the statistically significant increase in the ratio of ventricular dry weight to final bodyweight in ISO-pretreated rats confirms the ability of this drug to induce true myocardial hypertrophy, as previously reported (Verdetti & Mezin 1980; Chang et al 1982; Hayes et al 1984; Knufman et al 1987; Nanoff et al 1989; Vleeming et al 1990; Trindade et al 1992; Lahlou & Pinto Duarte 1998; Lahlou et al 2000). However, development of cardiac oedema in association with hypertrophy cannot be excluded, since the ratio of ventricular wet weight to bodyweight in the ISO-pretreated group was greater than the ratio of ventricular dry weight to bodyweight (data not shown).

It is well known that the activation of myocardial β-adrenoceptors, by both neurally-released noradrenaline and adrenaline released from the adrenal glands, results in both positive inotropic and chronotropic responses (Kaumann 1989). This study shows that 10-day ISO pretreatment decreased responsiveness to a β-adrenoceptor-mediated increase in LVSP and HR. This loss of responsiveness or desensitization is reflected in the increase of approximately 32- and 4-fold in the EC50 of ISO-induced changes in LVSP and HR, respectively. Such functional changes are in good agreement with previously reported data (Yamaguchi et al 1981; Chang et al 1982; Cabral & Vasquez 1984; Hayes et al 1984; Nanoff et al 1989; Vleeming et al 1990; Lahlou & Pinto Duarte 1998; Lahlou et al 2000). Hence, in view of its morphological and functional effects, the ISO rat model used in this study appears to be valid. We did not attempt to assess the mechanism underlying the observed loss in functional responsiveness after chronic ISO pretreatment. This mechanism has been extensively studied elsewhere, and has been shown to involve functional uncoupling of the β-adrenoceptor from the adenylate cyclase system and reduction of cardiac β_1-adrenoceptor density (Yamaguchi et al 1981; Chang et al 1982; Harden 1983; Nanoff et al 1989; Sibley & Lefkowitz 1985, 1987). In this study, baseline values of LVSP in Langendorff preparations were significantly higher after ISO pretreatment, as reported previously (Verdetti & Mezin 1980; Vleeming et al 1990; Lahlou & Pinto Duarte 1998; Lahlou et al 2000). As previously discussed (Vleeming et al 1990; Van Wijngaarden et al 1992), several factors might contribute to these ISO-induced changes in baseline parameters, such as an increase in ventricular wall stretch, resulting in elevated adenylate cyclase activity, an increase in coronary flow leading to an increase in myocardial function and oxygen consumption probably through a local Frank-Starling effect, or an increase in levels of intracellular calcium after termination of ISO pretreatment. However, the latter possibility is not supported by the finding of decreased basal adenylate cyclase activity after ISO pretreatment (Nanoff et al 1989; Sugiyama et al 1991).

The purpose of this study was to further corroborate the hypothesis that cardiac α_1-adrenoceptor stimulation maintains myocardial responsiveness under conditions in which cardiac β-adrenoceptor function is impaired. In Langendorff hearts from saline-pretreated rats, PHE infusion evoked a concentration-dependent increase in LVSP without significantly affecting the cardiac rate. This positive inotropic response is attributed to activation of cardiac α_1-adrenoceptors, since it can be competitively inhibited by selective α_1-adrenoceptor antagonists, such as doxazosin (Mertens et al 1992) and prazosin (Skomedal et al 1980). However, since PHE is a less selective α_1-stimulant, which is know to possess some affinity for β_1-adrenoceptors ($\alpha_1 > \beta_1$), a putative contribution of the latter receptors in the mediation of inotropic response to PHE could not be discounted (Skomedal et al 1988). The idea of such partial mediation was corroborated by this study, since in the presence of PROP, the apparent pD_2 for PHE-induced increase in LVSP shifted from 8.08 to 7.20 in preparations from saline-pretreated rats. After 10-day ISO pretreatment, the inotropic response to PHE was significantly enhanced over the whole concentration range used, leading to a shift to the left of the curve for the PHE-induced percentage increase in LVSP. Sensitivity to PHE, as indicated by the apparent pD_2, was enhanced 100-fold in ISO-pretreated hearts compared with saline-pretreated hearts. This enhancement is in good agreement with data obtained by Butterfield & Chess-Williams (1993), who studied the responsiveness to α_1-adrenoceptor stimulation in papillary muscle preparations from rats pretreated with ISO (40 μg kg^{-1}) daily for 3 days.

Partial mediation of the inotropic response to PHE by β-adrenoceptors was also observed in preparations from ISO pretreated rats. This conclusion is supported by the findings that under β-adrenoceptor blockade with PROP, the concentration-dependent increases in LVSP elicited by PHE were significantly attenuated compared with those obtained in the absence of PROP. Such results raise the possibility that the partial β-adrenergic mediation may be involved in the enhance-

ment of α_1-adrenoceptor stimulation after ISO pretreatment. To verify this possibility, inotropic responses to PHE under β-adrenoceptor blockade with PROP were compared between saline- and ISO-pretreated rats. A two-way analysis of variance showed that the curve of PHE-induced inotropic responses still remained shifted to the left after 10-day ISO pretreatment. This enhancement was of the same order of magnitude as that observed in the absence of PROP, since the difference in pD_2 values between ISO- and saline-pretreated rats in the absence of PROP (10.10 vs 8.08, respectively) was maintained under β-adrenoceptor blockade with PROP (9.20 vs 7.20, respectively). These results favour the hypothesis that the enhanced inotropic responses to PHE, after ISO-induced cardiac hypertrophy associated with β-adrenoceptor desensitization, is mediated via α_1-adrenoceptors. Taken together, these results may support the concept that α_1-adrenoceptor stimulation plays an important role in cardiac inotropy in rats, although this effect is much weaker in man. They further corroborated the hypothesis that cardiac α_1-adrenoceptors may act as a reserve mechanism to maintain myocardial responsiveness under certain conditions in which cardiac β-adrenoceptor function is impaired (Brückner et al 1985; Osnes et al 1985; Homcy et al 1991; Butterfield & Chess-Williams 1993). Since PROP treatment may induce additional impairment of β-adrenoceptor responsiveness, one might have expected that, in preparations from 10-day ISO pretreated rats, the enhanced inotropic responses to PHE would have been greater under β-adrenoceptor blockade compared with those obtained in the absence of PROP. However, this was not the case. It is possible that putative differential effects of PROP on cardiac parameters in saline- and ISO-pretreated rats may have contributed to this lack of correlation. This seems unlikely, however, since maximum percentage decreases in baseline LVSP and HR were of the same order of magnitude, irrespective of whether the rat was pretreated chronically with ISO or not.

This study did not attempt to assess the mechanism involved in the increased cardiac α_1-adrenoceptor sensitivity after chronic ISO pretreatment. This mechanism, which is not completely clear, may involve either a change in number and affinity of cardiac α-adrenoceptors, or alterations in post-receptor events. In this study, the maximum inotropic response to PHE was almost 38% greater in hypertrophied hearts, a finding that may be explained by an increased number of myocardial α_1-adrenoceptors. However, although ISO pretreatment has been reported to increase ventricular α_1-adrenoceptor-mediated responses to PHE, it did not enhance ventricular [^{3}H]prazosin binding sites (Butterfield & Chess-Williams 1993). Thus, the mechanism involved in the ventricular α_1-adrenoceptor supersensitivity must be of post-receptor origin, implying an altered signal transduction. Cardiac β-adrenoceptor activation generates the second messenger, cyclic AMP, whereas cardiac α_1-adrenoceptor stimulation is known to utilize changes in intracellular free calcium as its primary pathway of signal transduction (Minneman 1988; Scholz 1989). α_1-Adrenoceptor agonists, such as PHE, induce a degradation of PIP_2, leading to IP_3 and DAG formation. In some tissues, interactions between the cyclic AMP and PIP_2 have been demonstrated. In platelets, increasing the level of cyclic AMP inhibits the formation of IP_3 (Watson et al 1984) and also inhibits phosphorylation by protein kinase C (Takai et al 1982). Furthermore, elevating cyclic AMP levels inhibits the formation of IP_3 induced by α_1-adrenoceptor stimulation in rat aorta (Manolopoulos et al 1991) and kidney (Neylon & Summers 1988). Thus, as previously suggested (Butterfield & Chess-Williams 1993), if such an interaction occurs in the heart, a putative depression of β-adrenoceptor–cyclic AMP system by ISO pretreatment might result in enhanced inotropic responsiveness to α_1-adrenoceptor stimulation in hypertrophied hearts of ISO-pretreated rats.

In summary, this study shows that 10-day ISO pretreatment induced the expected cardiac β-adrenoceptor desensitization while simultaneously enhancing the positive inotropic responsiveness to PHE in Langendorff heart preparations. Hence, these findings support the hypothesis that cardiac α_1-adrenoceptors may act as a reserve mechanism to maintain myocardial function under conditions in which cardiac β-adrenoceptor function is compromised.

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