Catecholamine-induced heart injury in mice: differential effects of isoproterenol and phenylephrine

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Summary. The involvement of catecholamines in stress-induced heart injury is well documented. However, the contribution of adrenergic receptor types is less understood. Both the profile of plasma marker enzyme activities (lactate dehydrogenase-1 and aspartate transaminase) and the distribution and morphology of the lesions observed in tissue sections of adrenaline-injected mice resembled those of stressed (restraint and cold exposed) mice. Next, we compared the effect of isoproterenol (ß-adrenergic agonist) and phenylephrine (α_1-adrenergic agonist) on both heart function and tissue injury. In Langendorff-perfused rat hearts, α_1-adrenergic receptors made a minor contribution to the tonic effect of adrenaline, as indicated by the lack of effect on the heart rate and the delayed negative inotropic effect of phenylephrine. However, in whole mice, phenylephrine but not isoproterenol, induced an increase of both lactate dehydrogenase-1 and aspartate transaminase activities. Hearts of phenylephrine-injected mice showed necrotic lesions in subendocardial areas of the left ventricle. In addition a scattered focal leukocyte infiltration around single apoptotic-like myocytes was observed in the ventricle wall. Hearts of isoproterenol-injected mice showed a similar number of apoptotic-like myocytes, but a much lower number of necrotic areas, than phenylephrine-injected animals. Our results suggest that the cardiotonic effect of catecholamines involves mainly the ß-adrenergic receptors. However, the acute catecholamine-induced heart injury involves mainly α_1-adrenergic receptors.

Key words: Heart, Injury, Adrenaline, Isoproterenol, Phenylephrine

Introduction

Acute stress episodes may induce heart injury. Thus, immobilization or restraint, alone or in combination with other stimuli, induce myocardial necrotic lesions in several species (Raab et al., 1964; Jönsson and Johansson, 1974; Downing and Chen, 1985; Matsuoka et al., 1998). Stress-induced injury results in the release of cytosolic enzymes to the blood (Meltzer, 1971; Arakawa et al., 1997), and catecholamines appear to be responsible for the effect of acute stress (Cruickshank et al., 1987; Arakawa et al., 1997).

The heart expresses both ß- and α_1-adrenergic receptors (Brodde and Michel, 1999). The effect of ß-adrenergic receptor activation is well established: the increase of both heart rate and force of contraction. The effect of α_1-receptor activation is more complex. It is usually described a biphasic or a triphasic effect: initial positive inotropy, followed by a transient negative and finally a more sustained positive inotropy without effect on chronotropy (Terzic et al., 1993). However, some reports describe that α_1-adrenergic receptor activation induces a long-lasting negative inotropy (Kissling et al., 1997; Heubach et al., 2002).

The contribution of either receptor type to the acute damage induced by catecholamines is less understood. While some reports suggest that ß-adrenergic receptors are responsible for stress-induced heart damage (Arakawa et al., 1997; Matsuoka et al., 1998), others concluded that α_1-receptors, but not ß-receptors, are involved in the effect of catecholamines (Downing and Chen, 1985). Here we studied the damaging effect of an acute dose of isoproterenol (non-specific ß-adrenergic agonist) or phenylephrine (non-specific α_1-adrenergic agonist) in the mouse heart.

Materials and methods

Male Wistar rats (300-350 g) and Swiss-CD1 mice

(35-40 g) (Harlan-Interfauna, Barcelona, Spain) were kept in our vivarium under controlled conditions for at least 10 days before use. The experimental procedures were approved by the Committee on Animal Care of the University of Barcelona and by the Autonomous Government of Catalonia.

Heart perfusion

Rats were anesthetized (sodium pentobarbital 60 mg/Kg prepared in phosphate-buffered saline (PBS) supplemented with 2.5 mg heparin/ml). Hearts were mounted in a Langendorff apparatus and perfused at 37°C with Krebs-Henseleit solution, containing 5 mM glucose and 1 mM carnitine, and maintaining a hydrostatic pressure of 60-80 mmHg. The heart was connected to an electronic tension transducer (UF1, Pioden) maintaining a diastolic tension of 1g. After 15 min of basal recording, adrenaline, isoproterenol or phenylephrine was infused through the aortic cannula. The infusion rate was adjusted to obtain a final concentration (after dilution with the perfusion buffer) of 10 μM. From the continuous tension record, the mean heart rate in each 20 s period was calculated. The first derivative of the tension register was obtained and the mean maximal value in 20 s periods was calculated (+dT/dt).

Experiments in mice

To study the effect of stress, mice were introduced into small flat bottom cylinders (restrainers) with adjustable head and tail gates (Panlab, Barcelona, Spain). This procedure resulted in an almost complete immobilization of the animals. Animals were maintained at 4°C for 1 h and then returned to individual cages at room temperature for recovery. In some experiments, mice received a single i.p. injection (60 μmol/Kg) of adrenaline, isoproterenol or phenylephrine dissolved in PBS. During the recovery period, mice had free access to water and food.

Samples

To obtain samples, mice were anaesthetized (sodium pentobarbital 60 mg/Kg). Blood was collected into heparinized syringes from the inferior vena cava. Blood plasma was obtained by centrifugation. α-Hydroxybutyrate dehydrogenase (LDH-1) and aspartate aminotransferase (AST) were determined by standard procedures (Roche, Mannheim, Germany; assay kits HBDH MRP1 and AST MRP1). The heart was immediately excised after bleeding, fixed in Carnoy and embedded in paraffin to obtain 7 μm-thick sections. These sections were stained with hematoxylin-eosin (HE) and used for histological examination.

Histopathological score

To quantify the effect of adrenergic agonists, we obtained 4 transverse sections (separation between sections: 150 μm). After HE staining, we photographed (400x magnification) 12-14 randomly selected areas of the endocardial zone and the wall of the left ventricle. Each photograph was scored 0-3 by two independent observers. (0) no affectation; (1) sections containing a single apoptotic-like myocyte surrounded by infiltrated leukocytes; (2) sections containing a small necrotic area (2-3 neighbor damaged myocytes surrounded by infiltrated leukocytes); (3) sections containing a large necrotic area (more than 3 neighbors severely injured myocytes with heavy infiltration of leukocytes with or without hemorrhage).

Statistical analysis

All results are the mean± S.E.M. of the number of 5-7 animals per group. The statistical significance of the differences was determined by one-way or two-way ANOVA and post-hoc Tukey's test, depending on the experimental design. In all experiments, differences were considered significant when p<0.05.

Results

In the first experiment we studied the time-course of
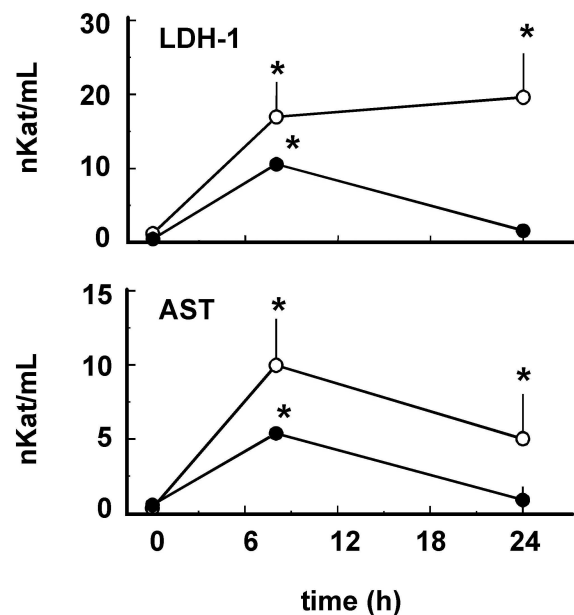
Fig. 1. Time-course of plasma enzyme activities after restraint and cold-exposure or adrenaline injection. At zero time, mice were (i) exposed to combined restraint and cold (4°C) for 1 h and then returned to their home cages (black symbols) or (ii) injected with adrenaline (60 μmol/Kg i.p.) (White symbols). At indicated times, some animals were sacrificed to obtain samples. Plasma lactate dehydrogenase-1 (LDH-1) and aspartate aminotransferase activities were determined. Results are the mean±S.E. of 5 animals per group. The statistical significance of the differences was determined by two-way ANOVA and post-hoc Tukey's test. *: p<0.05 vs corresponding zero time value.
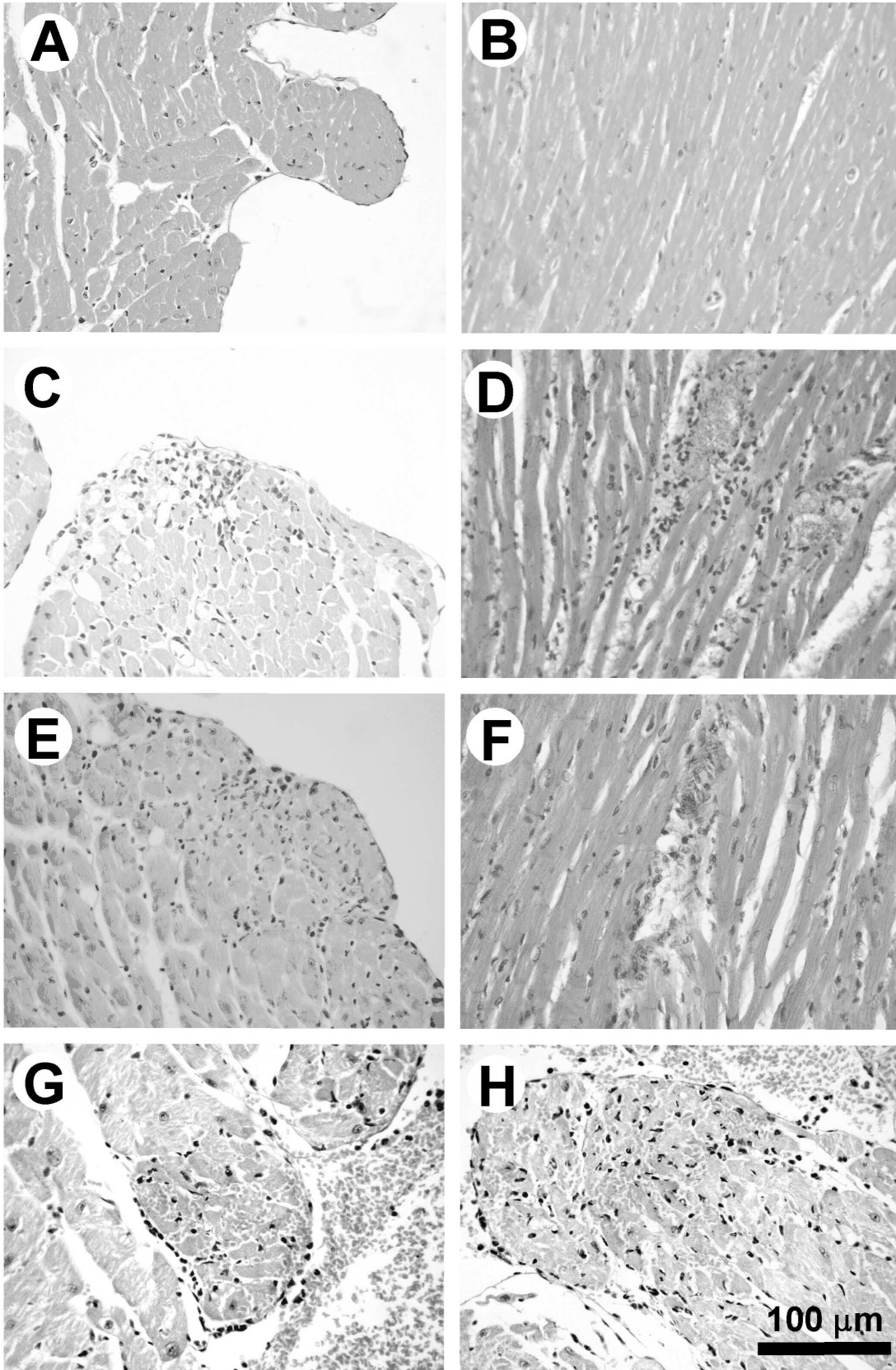
Fig. 2. Morphological evidence of necrotic lesions in restraint and cold-exposed and in adrenaline-injected mice hearts. Transverse sections (7 μm thick) of paraffin embedded hearts were stained with hematoxylin-eosin. Control mice: panels **A and B**. They correspond to representative images of the left ventricle endocardium **(A)** and the left ventricle wall **(B)**. Twenty-four h after exposure to stress: panels **C and D**. Representative images of the necrotic lesions observed in the left ventricle subendocardial areas **(C)** and in the left ventricle wall **(D)** are shown. Twenty-four h after adrenaline injection: panels **E to H**. Representative image of lesions observed in the left ventricle wall **(F)**. **Panels E, G and H** show images of the different degree of injury observed in adrenaline-injected mice.

marker enzyme activities in plasma of either restraint-cold stressed or adrenaline-injected mice. On the basis of preliminary dose-response experiments, an adrenaline dose of 60 μmol/Kg was chosen. At this dose, 80% mice survived for at least 24 h. Eight hours after the treatment, plasma LDH-1 and AST activities had increased up to similar levels in both adrenaline-injected and stressed mice (Fig. 1). After that time, plasma enzyme activities decreased in stressed mice, but remained high in adrenaline-injected mice.

The observation of HE stained sections of hearts 24 h after the treatment showed the existence of inflammatory lesions in both adrenaline-injected and stressed mice (Fig. 2). In stressed mice we observed scattered focal necrotic lesions both in the endocardial areas of the left ventricle (Fig. 2C) and in the left ventricle wall (Fig. 2D). In adrenaline-injected mice we observed both mild (Fig. 2E, F) and heavy (Fig. 2G,H) lesions. Heavy lesions resulted in endocardial wall hemorrhage, dense leukocyte infiltration and necrotic
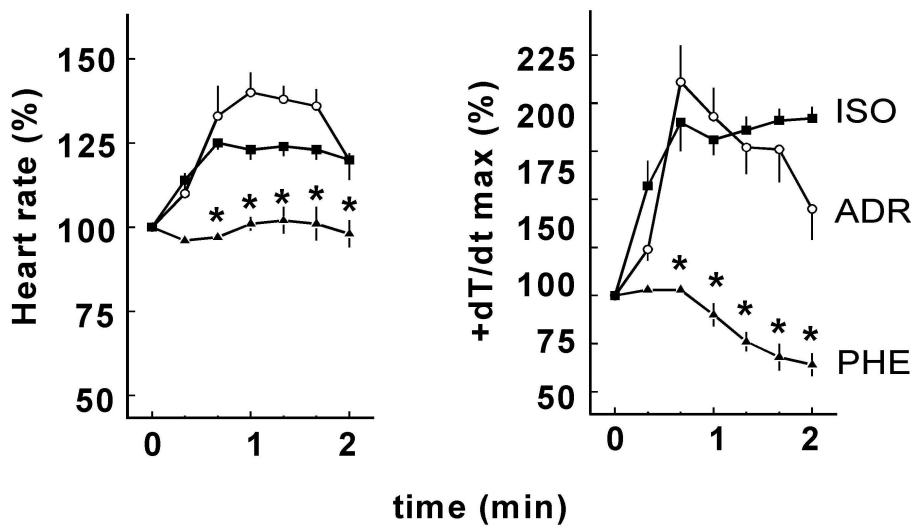
Fig. 3. Chronotropic and inotropic effects of catecholamines in Langendorff-perfused rat hearts. Perfused rat hearts were maintained for 15 min to record basal function parameters (referred to as 100%). Then (zero time), adrenaline (white circle), isoproterenol (black square) or phenylephrine (black triangle) were infused through the aortic cannula at 40 nmol/min. From the continuous tension record, the mean heart rate in each 20 s period was calculated. The first derivative of the tension function was obtained and the mean of maximal values in 20 s periods was calculated (+dT/dtmax). Results are mean±S.E. of % of basal value (n=5). The statistical significance of the differences was determined by two-way ANOVA and post-hoc Tukey's test. *: p<0.05 vs corresponding adrenaline value.
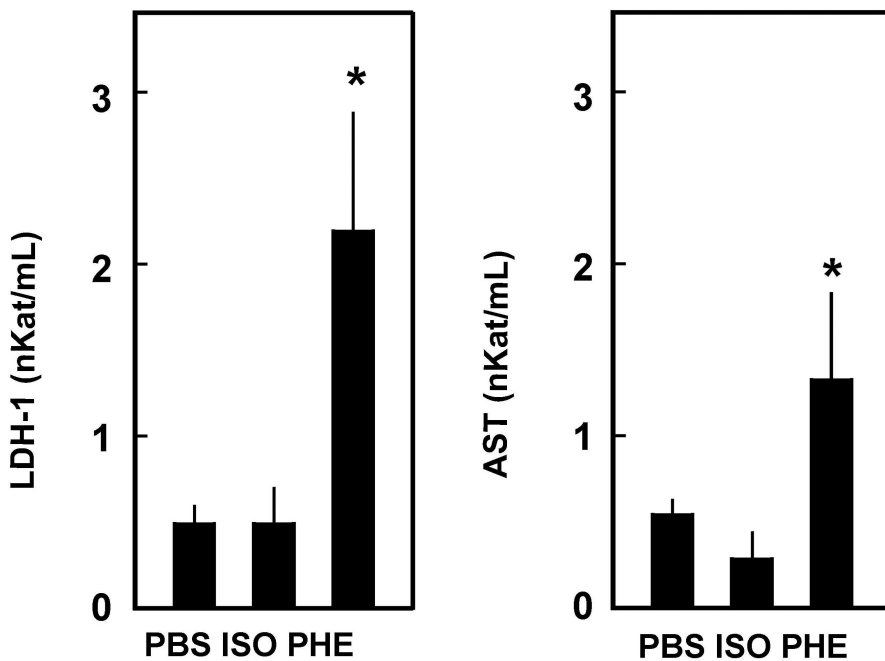

Fig. 4. Effect of adrenergic agonists on plasma enzyme activities. Mice were injected with isoproterenol (ISO) or phenylephrine (PHE) (60 μmol/Kg i.p.). Control mice were injected with PBS. Twenty-four h afterwards, samples were obtained to measure plasma LDH-1 and AST. Results are the mean±S.E. of 5 animals per group. The statistical significance of the differences was determined by one-way ANOVA and post-hoc Tukey's test. *: p<0.05.

myocytes. A large amount of erythrocytes and other blood cells were observed inside the left ventricle of the heavily injured hearts. There was a close relationship between the plasma enzyme activities and the degree of the injury observed in histological sections.

To study the effect of the selective activation of α_1-
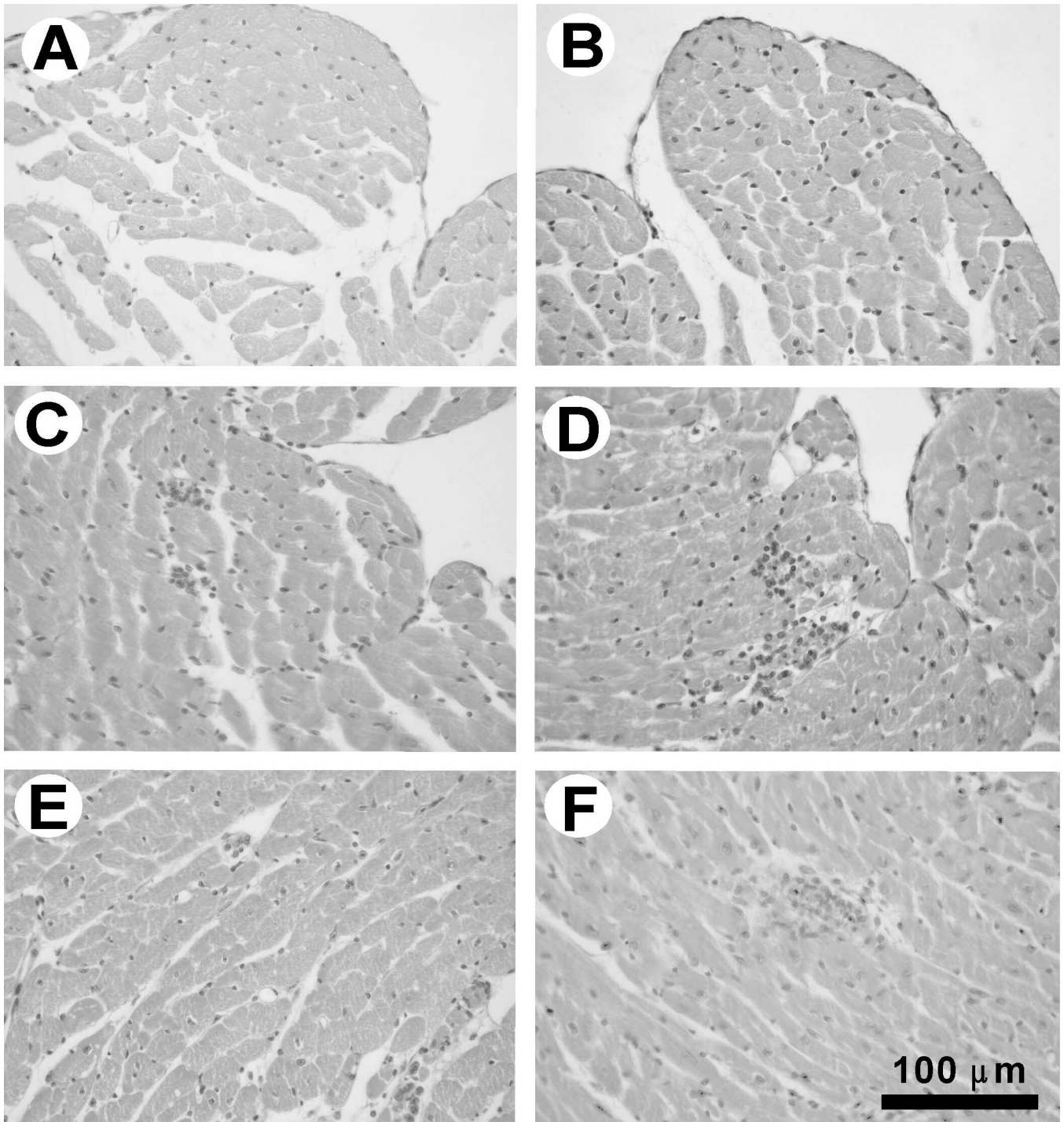
Fig. 5. Morphological evidence of heart injury in isoproterenol-injected mice. Twenty-four h after isoproterenol injection (60 μmol/kg i.p.) Hearts were obtained and processed for histological examination. **Panels A and B** show representative images of the left ventricle endocardial area. We did not observe evidence of necrotic lesions. In some animals we observed near the endocardium, leukocyte infiltration around single cells (**panel C**) or a small group of cells (**panel D**). In addition, similar focal leukocyte infiltration in deeper layers of the left ventricle wall was observed (**panels E and F**).
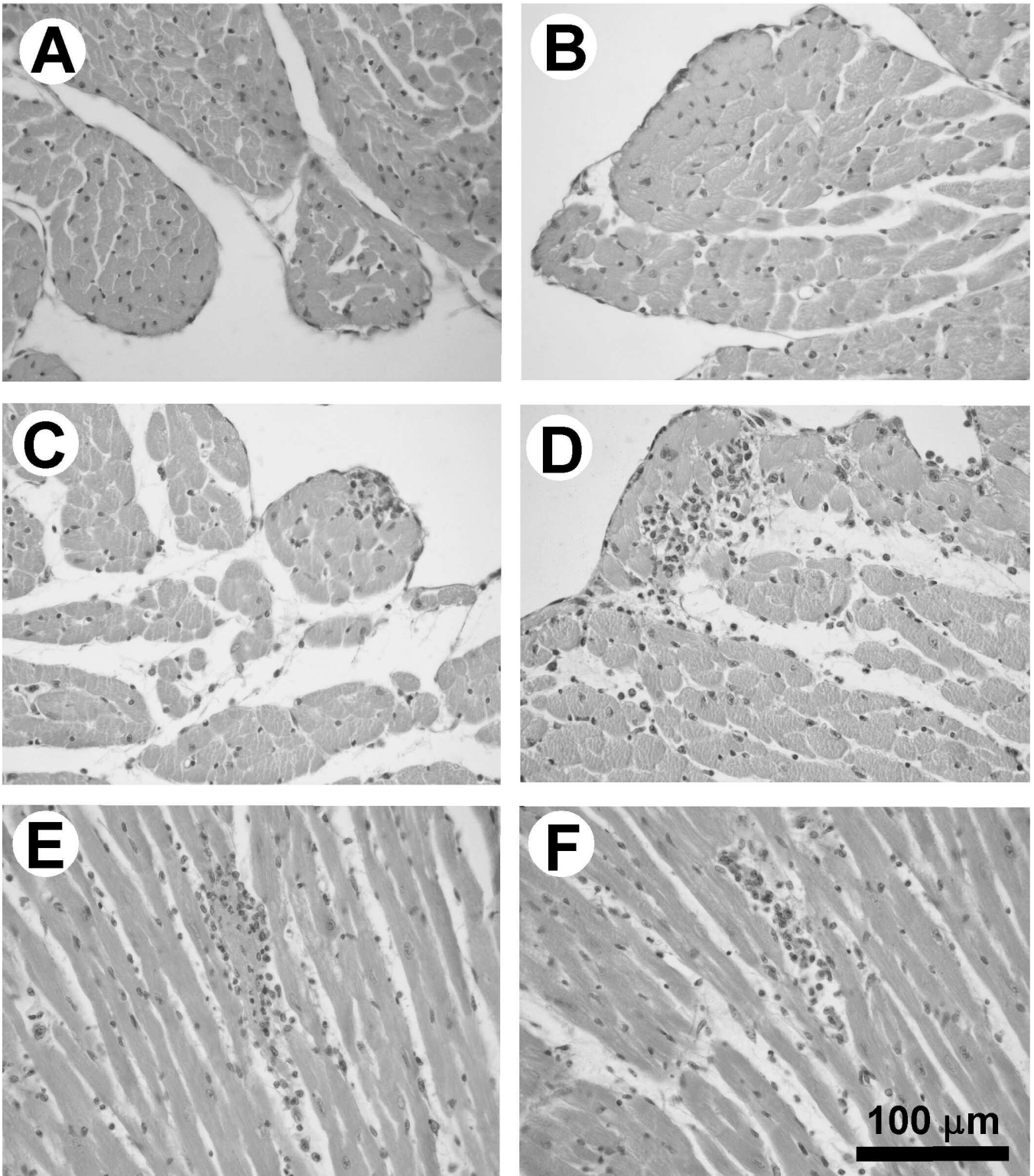
Fig. 6. Morphological evidence of heart injury in phenylephrine-injected mice. Twenty-four h after phenylephrine injection (60 μmol/kg i.p.) Hearts were obtained and processed for histological examination. Although some animals did not shown alterations in the endocardial area of the left ventricle (**panels A and B**), most mice showed small (**panel C**) or large (**panel D**) necrotic lesions. In addition, focal leukocyte infiltration around single myocytes in deeper layers of the left ventricle wall was observed (**panels E and F**).
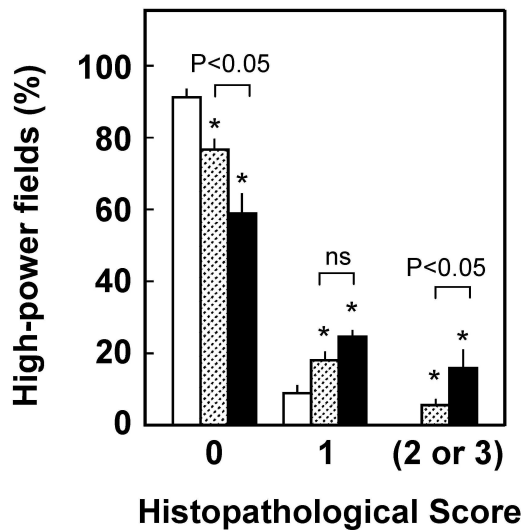
Fig. 7. Histopathological score of hearts from adrenergic agonist-injected mice. Histopathological score (0-3) was determined as indicated in methods. This figure shows the percentage of high-power fields (x 400 magnification) scored 0 (no affectation), 1 (containing a single apoptotic-like myocyte), and 2 or 3 (containing necrotic areas). White bars: control; dotted bars: isoproterenol-injected mice; black bars: phenylephrine-injected mice. Results are the mean±S.E. of 5 animals per group. The statistical significance of the differences was determined by one-way ANOVA and post-hoc Tukey's test. *: p<0.05.

or ß-adrenergic receptors, we analyzed first the effect of the infusion of either phenylephrine or isoproterenol on heart function in Langendorff-perfused rat hearts. Both adrenaline and isoproterenol increased the heart rate (Fig. 3). However, phenylephrine did not alter the heart rate. Adrenaline rapidly increased the contraction force as shown by the increase in +dT/dtmax. The highest value was obtained 40 s after the infusion started. Thereafter, contraction force progressively decreased. Isoproterenol produced a more sustained effect on contraction force. Phenylephrine showed no immediate effect on contraction force, but after 40 s this parameter decreased progressively.

Then, we studied the heart injury induced by the selective activation of α_1- or ß-adrenergic receptors. 24-h after agonist injection, enzyme markers of heart injury (LDH-1 and AST) were increased in plasma of phenylephrine-injected mice (Fig. 4). Isoproterenol did not affect these enzyme activities. Nevertheless, isoproterenol induced heart damage. In Fig. 5 we show the most significant alterations found in the heart of isoproterenol-injected mice. Contrarily to what we observed in adrenaline-injected or stressed mice, isoproterenol did not induce large necrotic lesions in the endocardium of the left ventricle (Fig. 5A,B). However, we observed in some animals focal infiltration of leukocytes around single injured-myocytes (Fig. 5C) or a small group of cells (Fig. 5D-F). In phenylephrine-injected mice, most animals showed focalized (Fig. 6C)

or disperse (Fig. 6D) infiltration of leukocytes in subendocardial area of the left ventricle. We also observed in phenylephrine-injected mice, focal infiltration of leukocytes around single injured-myocytes deeper in the ventricle wall (Fig. 6E,F).

To compare the effect of both adrenergic agonists, we scored (0-3) 48 to 50 areas of the left ventricle wall and endocardial zone from each animal. As shown in Fig. 7, most sections (92%) in control mice scored 0, and only 8% contained a single damaged myocyte (scored 1). In isoproterenol-injected mice, the number of areas scored 1 increased to 18%, and only 5% contained small or large necrotic areas (scored 2 or 3). In phenylephrine-injected mice, the number of areas scored 1 was similar to that in isoproterenol-injected animals (25%), but the number of photographs containing necrotic areas increased to 16%.

Discussion

The contribution of catecholamines to stress-induced heart injury has been recognized for more than 40 years (Raab et al., 1968). Indeed, lesions produced by catecholamines resemble those induced by stress (Todd et al., 1985a). Our results (plasma enzyme activities and histopathological analysis of heart sections) are in keeping with such role of catecholamines. LDH-1 is the most abundant LDH isoform in the heart, and a rise in LDH-1 activity in plasma was associated with heart injury (Crandall et al., 1981; Nirmala and Puvanakrishnan, 1996; Pareja et al., 2003). Also, the rise in AST has been associated with heart damage (Bleuel et al., 1995; Sánchez et al., 2002).

The heart of most mammals contains a much larger number of ß- (mostly of the ß1 subtype in the ventricle) than α_1-adrenergic receptors, and the contribution of the ß-adrenergic receptors to the cardiotonic effect catecholamines is well recognized (Brodde and Michel, 1999). Our results in the Langendorff-perfused heart system clearly indicate that the effect of adrenaline is mostly mediated by ß-adrenergic receptors, as shown by the similarity of the effect of adrenaline and isoproterenol.

The effects of α_1-adrenergic receptor in the heart have been controversial. Although many reports indicate a positive inotropic effect, others have reported negative or bi/triphasic effects (for review see (Terzic et al., 1993)). Moreover, opposite effects have been reported in left and right ventricle trabeculae (Wang et al., 2006). Our results suggest that α_1-adrenergic stimulation may be responsible for the progressive decline in the inotropic response to adrenaline. It is worth noting that while adrenaline induced a maximal inotropic response 40 s after stimulation and decreased thereafter, the ß-agonist isoproterenol produced a sustained inotropic effect. The decline observed 40 s after adrenaline stimulation may be explained by the α_1-receptor mediated negative-inotropy. Note that the negative-inotropic effect of phenylephrine was delayed 40 s.

Indeed, α_1-adrenergic receptors do not contribute to the chronotropic effect of adrenaline. This was already recognized (Terzic et al., 1993).

The contribution of ß-adrenergic receptors to stress-induced heart damage was suggested by the use of receptor blockers (Cruickshank et al., 1987; Arakawa et al., 1997). In addition, isoproterenol (at high or repeated doses) was used to study catecholamine-induced myocardial necrosis (Judd and Wexler, 1969; Wexler, 1970; Todd et al,. 1985b; Tipnis et al., 2000). Our results indicate that the sole stimulation of ß-adrenergic receptors does not reproduce the effect of adrenaline on heart injury. The small lesions observed (recruitment of leukocytes on single or few cells) is compatible with the known effect of ß-agonists inducing apoptosis both in cultured myocytes (Craommunal et al., 1998) and in the whole animal (Shizukuda et al., 1998). In the whole animal, neutrophil recruitment occurs early after the induction of apoptosis (Lawson et al., 1998).

Concerning the involvement of α_1-adrenergic receptors in catecholamine-induced heart damage, Downing and Chen (Downing and Chen, 1985) observed that the blockade of these receptors with phentolamine reduced the extent of heart injury caused by endogenous (tyramine-induced) catecholamine release. Our results agree in that α_1-adrenergic receptors play an important role in catecholamine-induced heart injury. The extent and the localization of the lesions observed in phenylephrine-injected mice resemble those induced by adrenaline or by restraint and cold-induced stress.

Phenylephrine activates α_1-receptors not only in the heart, but also in the vascular system, where it induces vasocontriction and thus, an increase in blood pressure. It is known that hypertension induces myocardial cell death and finally remodeling and hypertrophy (Wakatsuki et al., 2004). Since our results suggest that α_1-adrenergic receptors are essential in the induction of necrotic heart damage, it is conceivable that the acute catecholamine-induced heart injury may involve the action of these hormones in both the heart itself and the vascular bed.

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