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Lusitropic effects of α- and β-adrenergic stimulation in amphibian heart

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Abstract

The effects of β and α-adrenergic stimulation in amphibian superfused hearts and ventricular strips were studied. Superfusion with 3×10^{-8} M isoproterenol produced a positive inotropic effect, as detected by a 92 ± 24% increase in the maximal rate of contraction ($+\dot{T}$) and a positive lusitropic effect characterized by a decrease in both the ratio $+\dot{T}/-\dot{T}$ (23 ± 5%) and the half relaxation time ($t_{1/2}$) (19 ± 4%). The mechanical behavior induced by the β-agonist was associated with an increase in the intracellular cAMP levels from control values of 173 ± 19 to 329 ± 28 nmol/mg wet tissue. Hearts superfused with ^{32}P in the presence of isoproterenol showed a significant increase in Tn I phosphorylation (from 151 ± 13 to 240 ± 44 pmol ^{32}P/mg MF protein) without consistent changes in phosphorylation of C-protein. In sarcoplasmic reticulum membrane vesicles, no phospholamban phosphorylation was detected either by β-adrenergic stimulation of superfused hearts or when phosphorylation conditions were optimized by direct treatment of the vesicles with cAMP-dependent protein kinase (PKA) and [γ ^{32}P] ATP.

The effect of α-adrenergic stimulation on ventricular strips was studied at 30 and 22°C. At 30°C, the effects of 10^{-5} to 10^{-4}M phenylephrine on myocardial contraction and relaxation were diminished to non significant levels by addition of propranolol. At 22°C, blockage with propranolol left a remanent positive inotropic effect (10% of the total effect of phenylephrine) and changed the phenylephrine-induced positive lusitropic effect into a negative lusitropic action. These propranolol-resistant effects were abolished by prazosin. Our results suggest that in amphibian heart, both the inotropic and lusitropic responses to catecholamines are mainly due to a β-adrenergic stimulation which predominates over the α-adrenergic response. Phospholamban phosphorylation seems not to be involved in mediating the positive lusitropic effect of β-adrenergic agents whereas phosphorylation of troponin I may play a critical role. (Mol Cell Biochem **141**: 87–95, 1994)

Key words: amphibian myocardial relaxation, α and β-adrenergic stimulation, Ca^{2+} myofibrillar sensitivity, protein phosphorylation

Introduction

In mammalian heart, the positive inotropic effect of α and β-adrenergic agents is coupled with different changes in relaxation. Whereas β-adrenergic agonists cause a relaxant (positive lusitropic) effect [1–3], α-adrenergic stimulation evokes a negative lusitropic action [4]. Although both interventions appear to increase the amplitude of cytosolic Ca^{2+} transient, they have opposite effect on myofilament responsiveness to Ca^{2+}. α-adrenergic agonists increase myofilament-Ca^{2+} sensitivity [5–7]. This increase, which has been postulated to contribute to their positive inotropic effect [5–7], might also explain their negative lusitropic action [4]. β-agonists decrease myofibrillar responsiveness to

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Ca^{2+} by enhancing the dissociation rate of Ca^{2+} from troponin C [8]. Although this effect may contribute to their positive lusitropic action [9], it seems now well established that phosphorylation of the sarcoplasmic reticulum (SR) protein phospholamban is the main mechanism mediating the relaxant effect of β-agonists in mammalian heart [10–14].

In amphibian heart, β-adrenergic agonists induce a positive lusitropic effect similar to that observed in mammalian heart [3, 5, 16]. The underlying mechanism of this action is, however, far from being understood. Different types of evidence indicate that amphibian ventricular relaxation is mainly determined by Ca^{2+} extrusion through the Na^{+}/Ca^{2+} exchanger [17]. However more recent studies lend support to the idea that, although sparsely distributed, the amphibian cardiac SR is able to store Ca^{2+} [18, 19]. Thus, the role of the SR and phospholamban phosphorylation in mediating the responses to β-adrenergic agents need to be considered. Previous studies of our laboratory have shown that dcAMP produces a decrease in the myofilament Ca^{2+} sensitivity of toad ventricular trabeculae [16, 20]. The mechanism underlying this decrease is unknown. An interesting hypothesis, related to the possible involvement of C-protein phosphorylation produced by β-agonists in toad myocardial relaxation has been suggested [21]. In this context, the role of the phosphorylation of other myofibrillar proteins like troponin I (Tn I) should be considered. The present investigation explores the possible contribution of phospholamban and myofibrillar proteins phosphorylation to the positive lusitropic effect of β-agonists in amphibian heart.

The effects of α-adrenergic stimulation on the amphibian heart are far less understood and they are still a matter of controversy. Whereas some authors have failed to find an α-adrenoceptor-mediated inotropic response [15, 22] others suggest that α-adrenergic receptors may participate in the positive inotropic action of sympathomimetic amines [23–25]. Moreover, nothing is known about the effect of α-adrenergic stimulation on amphibian ventricular relaxation. The second aim of this investigation is to verify the existence of an α-adrenergic-mediated inotropic response in the amphibian heart and to ascertain whether this response is associated with any effect on myocardial relaxation and myofibrillar sensitivity to Ca^{2+}.

Materials and methods

Twenty two ventricular strips and thirteen superfused hearts from adult toads (Bufo arenarum Hensel) were used throughout this study. All animals were kept in the laboratory at room temperature (22–26°C) with free access to water. At the moment of the experiment the animals were pithed and the hearts rapidly excised.

Ventricular strips

The strips were prepared from circular cuts through the ventricle. The length of the preparations ranged between 5 and 8 mm and their largest diameter was not greater than 1 mm. The methods used for mounting, stimulation and recording were essentially identical to those reported previously [3]. Briefly, the ventricular strips were mounted vertically in chambers containing Ringer solution in equilibrium with a gas mixture of 95% O_2 and 5% CO_2. The composition of the Ringer solution was in mM: 115 NaCl, 3.2 KCl, 1.35 $CaCl_2$, 20.6 $NaHCO_3$, 0.3 NaH_2PO_4, 1.2 $MgCl_2$, 13 glucose and 0.04 Na_2 EDTA. The pH was kept constant at 7.4 and the temperature was maintained at 30°C in one set of experiments and at 22°C in another. Rectangular pulses of 10 msec duration with an amplitude 20% higher than the threshold of each preparation were delivered from an electronic stimulator. Contraction frequency was kept constant at 12 beats/min. Records of developed tension (DT) and its first derivative ($\dot{T}$), were obtained at high paper speed on a Gould 2400 recorder. After mounted, the preparations were allowed to stabilize for 1 h. After this period, the strips were gradually stretched until they reached the length at which maximal tension occurred (Lmax). This length was maintained throughout the experiment. Cumulative concentration-response curves to phenylephrine (from 10^{-9} to 10^{-4} M) were obtained at 30°C or 22°C in the absence or presence of adrenergic blocking agents (10^{-6} M propranolol plus 10^{-6} M prazosin). Blocking agents were added immediately after the stabilization period and their effects were monitored for half an hour to allow for stabilization of any possible effect of the drugs on contractility or relaxation.

Isolated hearts

Toad hearts were superfused with the same Ringer solution described for the ventricular strips at 30°C. The heart was attached via the right aorta to a cannula, excised and connected to the perfusion equipment. Left aorta was clamped. Aortic flow (4 ml/min) and heart rate (100 beats/min) were kept constant through the experimental procedure. Records of DT and $\dot{T}$ were measured in a segment of the ventricular wall by sewing to it an isometric strain gauge arch (Micro measurements type MA-06-030LB-120). The initial length of the isometric gauge was set by stretching the segment sewn by approximately 30%. To measure ^{32}P incorporation into phosphoproteins, the hearts were perfused for 10 min without recirculation with Ringer solution, and then for 60 min with recirculation with the same buffer containing 10 μCi/mi of ^{32}P. After perfusion with ^{32}P the circuit was returned to the initial conditions, and the hearts were perfused during the next 4 min with Ringer solution either in the

absence (control) or presence of 3×10^{-8} M isoproterenol. At the end of each experiment, the ventricle was quickly frozen with a Wollenberger clamp precooled in liquid nitrogen, pulverized and stored at –70°C until further assays. To compare the amphibian phosphorylation pattern with that of mammalian, a group of isolated rat hearts were perfused as previously described [14].

SR membrane vesicles and myofibrils isolation

SR-membrane vesicles were prepared from individual frozen hearts as previously described [14] except that the two last spins (45000 g) were carried out for 60 min. Protein was measured by the method of Bradford having albumin as standard. The yield was 1 mg membrane protein/g of cardiac tissue. Myofibrils (MF) were prepared from the first pellet generated in the fractionation of microsomal vesicles as described by Robertson *et al.* [8].

Phosphorylation of isolated SR membrane vesicles and myofibrils

SR-membrane vesicles (1 mg/ml) and MF fractions isolated (1 mg/ml) from nonperfused hearts were phosphorylated in a medium containing 50 mM Tris-HCl (pH 7), 2 mM $MgCl_2$, 5 mM EGTA and 5 U/ml catalytic subunit of PKA. The reaction was started by addition of 50 μM of [γ-^{32}P]ATP. After incubation for 2 min at 30°C, the reaction was terminated by addition of the sample buffer used for electrophoresis (see below).

SDS-polyacrylamide gel electrophoresis

Polyacrylamide-gel electrophoresis of microsomal and myofibrillar proteins was performed as described by Porzio and Pearson [26], using 10% polyacrylamide gels. SR and myofibrillar samples were solubilized by addition of an equal volume of sample buffer containing 2% SDS, 62.5 mM Tris-HCl (pH 6.8), 10% glycerol and 5% β-mercaptoethanol with a trace of bromophenol blue as a tracking dye. Gels were stained with solution containing 7% acetic acid, 40% methanol and 0.25% Coomassie Blue and destained with the same solution without addition of the dye. Autoradiography was performed by placing the gels, previously dried under vacuum at 90°C, in contact with 3 MXD film with a Kodak Min-R intensifying screen. The exposure time was 14 days at –70°C. Phosphate incorporation into phosphoproteins was calculated as pmol ^{32}P/mg SR or myofibrillar protein based on the specific activity of ^{32}P in phosphocreatine determined in each heart, according to Barron *et al.* [27]. The specific activity of ^{32}P-phosphocreatine was approximately 10^6 cpm/μmol.

cAMP assay

Myocardial cAMP content was determined by using a radioimmunoassay kit (Du Pont-NEN) as previously described [14] in hearts perfused with the same protocol described above but without addition of ^{32}P. Results were expressed as nmol cAMP/mg wet tissue.

Skinned trabeculae

EGTA-treated trabeculae (mean length: 935 ± 145 μm, mean diameter: 190 ± 14 μm) were prepared and mounted as previously described [16, 20]. The trabeculae were soaked for 24 h in a disruption solution containing in mM: 140 K propionate, 2 Mg acetate, 10 EGTA, 5 ATP disodium salt, brought at pH 7.2 with KOH. After being treated with EGTA, the trabeculae were mounted horizontally in a muscle bath between an adjustable stainless steel rod and a fixed tension transducer (AE801 Horten, Norway). To prevent dispersion of the data due to the length-dependence of Ca^{2+} sensitivity of the myofibrils described in mammalian heart [28], special care was taken to stretch each preparation approximately 30% of their slack length. Temperature of the bath was kept constant at 22°C. Contraction and relaxation of the trabeculae were induced by superfusion with 'activating' or 'relaxing' solutions. Relaxing solution contained in mM: 140 KCl, 7 $MgCl_2$, 5 ATP, 15 phosphocreatine, 10 EGTA and 25 imidazole and 0.1 mg/ml creatine kinase, pH 7.0. The calculated ionic strength of the relaxing solution was 0.180 M. The activating solution had the same composition, except that some or all of the EGTA was replaced by 4–10 mM Ca-EGTA. The free ionic concentrations in relaxing and activating solutions were calculated using a computer program [29]. After a stabilization period of 20 min in the relaxing solution, the trabeculae were incubated for 10 min with 10^{-6} M atenolol in the same relaxing solution. Thereafter all the activating and relaxing solutions contained atenolol 10^{-6} M. Then the trabeculae were made to contract at pCa 6.25 and then to relax at pCa 9.0. This procedure was repeated in the same trabeculae after a 10 min incubation period with phenylephrine 10^{-4} M. Preliminary experiments have shown that pCa 6.25 is approximately the ED_{50} of the tension-pCa curve [16, 20].

Statistical analysis

The depicted values are averages ± SEM. The statistical significance was obtained by Student *t* test for paired or non

paired samples as appropriate. $P < 0.05$ was considered to reflect significant differences.

Results

β-adrenergic stimulation in amphibian heart

Superfusion with 3×10^{-8} M isoproterenol induced a positive inotropic effect, as detected by a 92 ± 24% increase in the maximal rate of contraction ($+\dot{T}$) and a positive lusitropic effect characterized by a decrease in both the ratio $+\dot{T}/-\dot{T}$ (23 ± 5%) and the half relaxation time ($t_{1/2}$) (19 ± 4%). The mechanical behavior induced by the β-agonist was associated with an increase in the intracellular cAMP levels from basal values of 173 ± 19 to 329 ± 28 nmol/mg wet tissue (Fig. 1). These results are in agreement with previous findings [16].

In mammalian myocardium, it is well known that phosphorylation of SR and myofibrillar proteins is the cellular mechanism responsible for the relaxant effect of β-adrenergic agents. In an attempt to study whether the same mechanism participates in the β-adrenergic induced positive lusitropic effect in amphibian myocardium, toad hearts were superfused with ^{32}P to detect phospholamban, Tn I and C-protein phosphorylation. Figure 2A shows an autoradiography of SR membrane vesicles obtained from toad hearts superfused with or without 3×10^{-8} M isoproterenol. The phosphorylation pattern obtained with toad hearts was compared with that of rat hearts perfused following the same protocol. No isoproterenol-induced ^{32}P incorporation into bands within Mr range of either the low and high molecular weight forms of phospholamban were detected in toad heart while in rat cardiac muscle the β-agonist produced the already described phosphorylation of phospholamban [13, 14]. In order to improve the specific activity of the phosphorylation reaction, isolated SR membranes were phosphorylated in the presence of [γ-^{32}P]ATP and PKA. To account for the possibility of a low ratio phospholamban/mg SR protein, increased amounts of toad SR membranes were phosphorylated. Again, no phospholamban phosphorylation in toad SR was detected (Fig. 2B).

Figure 3A shows an autoradiography of MF obtained from toad hearts superfused with ^{32}P. Isoproterenol produced an increase in Tn I phosphorylation whereas phosphorylation of C-protein is less evident. The quantitative overall results of these experiments indicate a significant increase in ^{32}P incorporation in Tn I 151 ± 13 to 240 ± 44 pmol ^{32}P/mg MF protein without statistically significant changes in C-protein phosphorylation. When isolated MF were phosphorylated

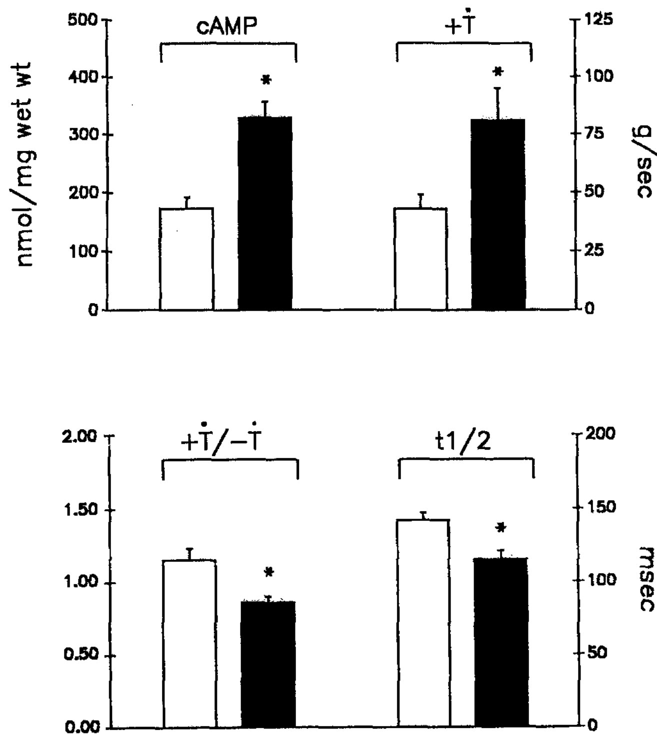

Fig. 1. Effects of β-adrenergic stimulation on mechanical activity and cAMP levels in isolated toad hearts. Superfusion with 3×10^{-8} M isoproterenol produces a positive inotropic effect (increase of maximal rate of contraction, $+\dot{T}$), and a positive lusitropic response (decrease of the ratio $+\dot{T}/-\dot{T}$ and the time to half relaxation, $t_{1/2}$). These mechanical responses were associated with an increase in intracellular cAMP levels. Bars represent means ± SE. Mechanical data, n=7. cAMP data, n=4. *$P \leq 0.05$.

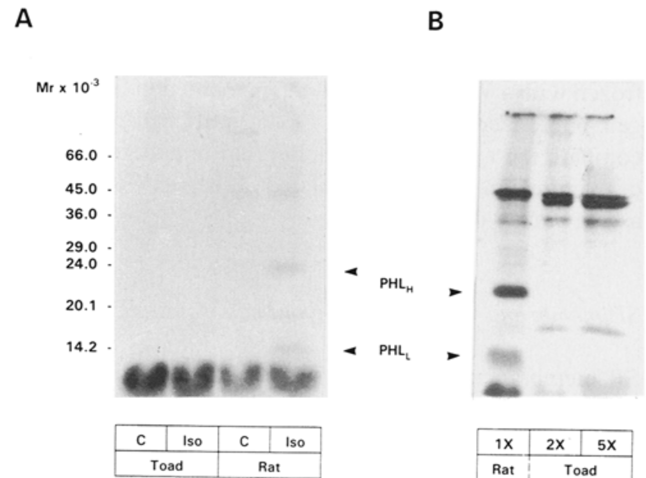

Fig. 2. Phosphorylation of SR membrane vesicles. A: Autoradiogram of toad and rat SR membrane vesicles isolated from ^{32}P perfused hearts with or without of 3×10^{-8} M isoproterenol. No isoproterenol induced ^{32}P incorporation into a band within the Mr range of phospholamban (PHL) was detected in toad heart while the same treatment in rat myocardium produced the already known phosphorylation of phospholamban. B: SR membrane vesicles phosphorylated by the catalytic subunit of cAMP-dependent protein kinase plus [$\gamma^{32}P$]-ATP. The lack of phospholamban phosphorylation was confirmed with increased amounts of toad SR protein (2× and 5×). C: control, Iso: 3×10^{-8} M isoproterenol, PHL_H: heavy phospholamban, PHL_L: light phospholamban.

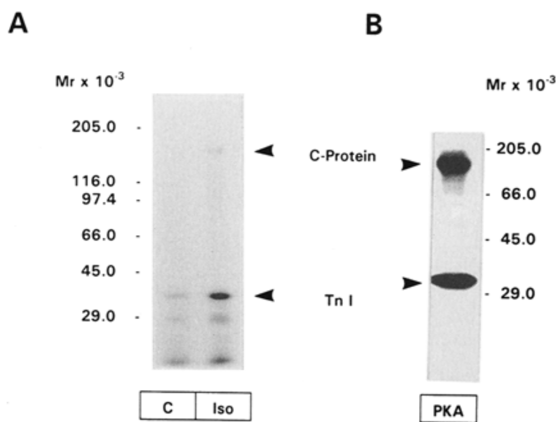

Fig. 3. Autoradiograms of toad isolated myofibrillar proteins from ^{32}P superfused hearts with (Iso) or without (C) the administration of 3×10^{-8} M isoproterenol (A); or from isolated myofibrils phosphorylated by the catalytic subunit of cAMP-PK (PKA) plus [γ^{32}P]-ATP (B). Isoproterenol and cAMP-PK enhanced both troponin I and C-protein phosphorylation.

with the catalytic subunit of PKA and [γ-^{32}P]ATP, two major phosphoproteins were detected (Fig. 3B). The Mr of these phosphoproteins correspond to those described for C-protein and Tn I in amphibian heart [21].

α-adrenergic stimulation in amphibian heart

Figure 4 illustrates the effect of phenylephrine administration on different mechanical parameters of the isometric twitch. All the experiments of this series were carried out at 30°C. 10^{-5} to 10^{-4} M phenylephrine produced a positive inotropic effect as shown by a singificant increase in DT and $+\dot{T}$ associated with a significant positive lusitropic effect characterized by a decrease in the ratio $+\dot{T}/-\dot{T}$. DT and $+\dot{T}$ in the presence of 10^{-4} M phenylephrine increased 43.5 ± 11.5% and 44.6 ± 7.8% respectively over control levels while the ratio $+\dot{T}/-\dot{T}$ fell 11.33 ± 3.87%. $t_{1/2}$ was also slightly but significantly decreased. When dose-response curves to phenylephrine were performed in the presence of the β-adrenoceptor antagonist propranolol (10^{-6} M) all the above mentioned inotropic and lusitropic changes were completely abolished. In the presence of propranolol 10^{-4} phenylephrine still produced a slight and non significant effect on DT and $+\dot{T}$. These results are consistent with a β-adrenoceptor-mediated effect of phenylephrine in toad myocardium.

Figure 5 shows the effect of phenylephrine on twitch mechanical parameters from a series of experiments performed at 22°C. This intervention is known to potentiate the α-adrenoceptor-mediated inotropic response [23, 30, 31]. Under these experimental conditions, the enhancement of DT and $+\dot{T}$ induced by phenylephrine in the presence of the β-blocking agent propranolol (10^{-6} M) although still small, attained statistically significant levels. This remanent effect accounts for approximately 10% of the total positive inotropic effect and was completely abolished by the α-adrenoceptor antagonist prazosin (10^{-6} M). The relaxation index $+\dot{T}/-\dot{T}$, which decreased in the presence of phenylephrine, as was the case at 30°C, shifted to values higher than control levels with the administration of propranolol. The same behavior was followed by $t_{1/2}$. This indicates a change from the typical relaxant effect of β-adrenergic stimulation to a negative lusitropic response characteristic of the α-adrenoceptor-mediated effect. Furthermore, as it was observed for DT and $+\dot{T}$, this negative lusitropic effect which could not be detected at 30°C, was readily abolished in the presence of prazosin. 10^{-6} M propranolol or 10^{-6} M propranolol plus 10^{-6} M prazosin before the dose-response curve to phenylephrine, did not significantly affect either contractility or relaxation parameters at any of the temperatures studied. The results indicate that a small but significant α-adrenergic mediated positive inotropic effect can be detected in toad ventricle at low temperature. As in mammalian ventricle, this effect is associated with a negative lusitropic action.

Figure 6A shows the results of a typical experiment in which Ca^{2+} responsiveness of the contractile system was evaluated. Perfusion with 10^{-5} M phenylephrine did not produce any significant change in the ability of the fiber to generate force at pCa 6.25. This lack of effect was not due to deterioration of the preparation during the second exposure to high Ca^{2+}, since, in agreement with previous findings [16], control experiments demonstrate that in the absence of drugs, the second contraction reproduces the first one (results not shown). Pooled results of the effect of phenylephrine on myofilament Ca^{2+} sensitivity are shown in Fig. 6B.

Discussion

β-adrenergic stimulation

In agreement with previous studies the present experiments show that β-adrenergic agonists increased contractile force, accelerated the rate of relaxation and enhanced intracellular cAMP levels in amphibian cardiac muscle [3, 15, 16]. β-adrenergic stimulation failed to elicit changes in phospholamban phosphorylation even when the phosphorylation conditions were optimized by direct phosphorylation of SR vesicles with PKA. Thus, in the amphibian myocardium phospholamban phosphorylation does not seem to be involved in mediating the responses to β-adrenergic agents. The absence of phospholamban in amphibian cardiac SR suggested by these results could be added to the list of structural [19] and functional [32, 33] differences found between mammalian and amphibian cardiac SR.

At the level of the myofibrils, phosphorylation of Tn I was

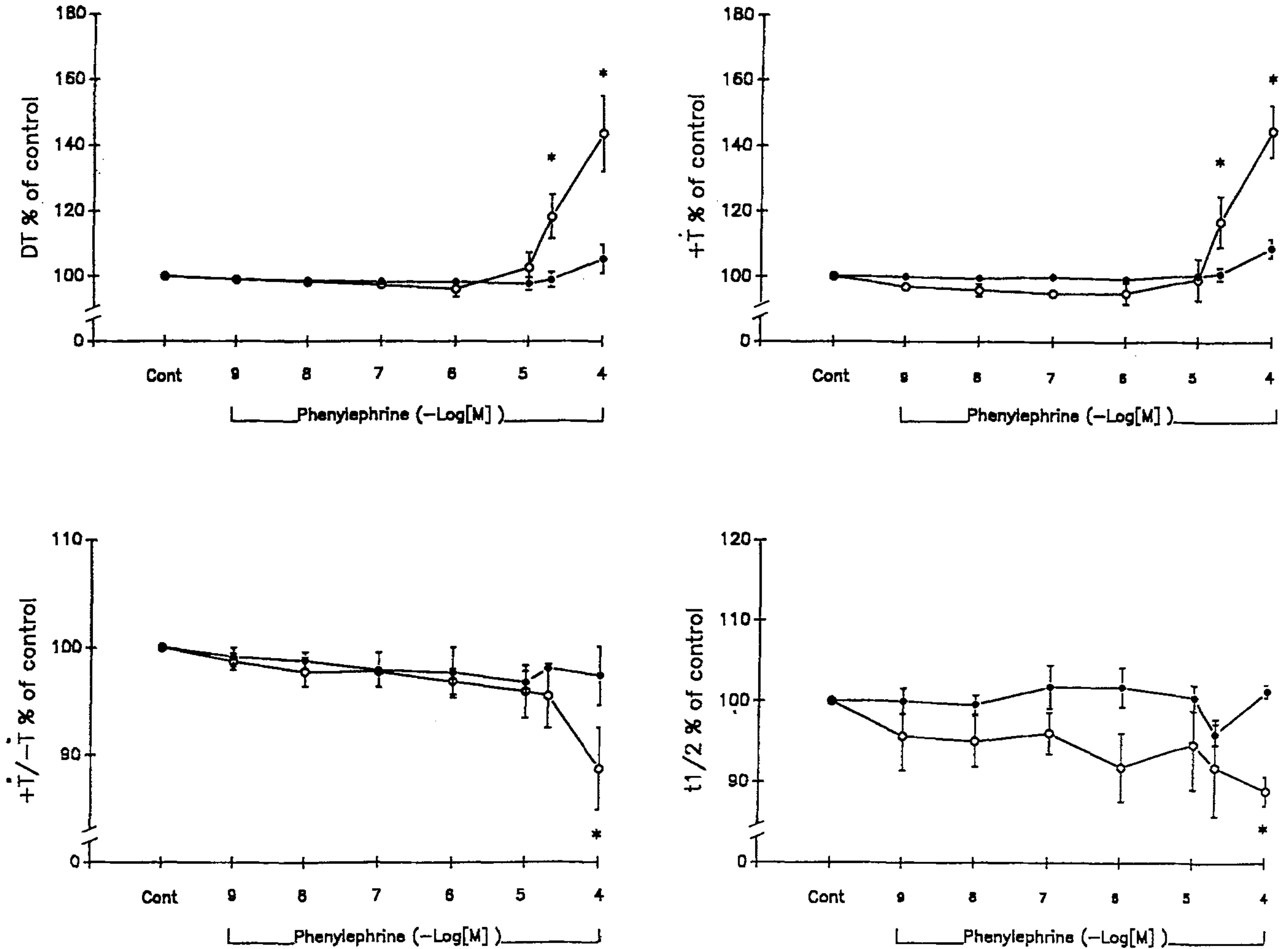

Fig. 4. Effects of phenylephrine on different parameters of the isometric twitch. Experiments were carried out at 30°C, in the absence of the presence of 10^{-6} M propranolol. The positive inotropic (increase in developed tension (DT) and maximal rate of contraction (+Ṫ)) and lusitropic (decrease in the ratio +Ṫ/-Ṫ and time to half relaxation ($t_{1/2}$)) effects induced by phenylephrine were completely abolished by the β-blocker. O: phenylephrine, ●: phenylephrine plus propranolol.

detected both in the superfused heart after isoproterenol administration and when the myofibrils were first isolated and then phosphorylated with PKA. Although C-protein phosphorylation was not consistently found in ^{32}P superfused hearts, phosphorylation of this protein could be detected when myofibrils were treated with catalytic subunit of PKA. Our results differ from those reported by Hartzell who has shown a consistent β-adrenergic induced C-protein phosphorylation in the intact toad heart with a variable phosphorylation ofTn I [21]. According to those findings, he suggested an important role of C-protein phosphorylation in the regulation of myocardial relaxation. The present results, although unable to discard the participation of C-protein proposed by Hartzell [21], strongly suggest that Tn I may also play a role in the decrease in Ca^{2+} sensitivity produced by increase in cAMP levels [16, 20], like in mammalian heart. In this specie, there are evidences that phospholamban phosphorylation plays a more dominant role than Tn I in the relaxant effect of β-adrenergic stimulation [10, 11]. However the relative contribution of myofibrillar protein phosphorylation to the relaxant effect of β-agonists might change under different physiological conditions [34]. In amphibian heart and in our experimental conditions, no phospholamban phosphorylation was detected. Therefore, the decrease in myofilament responsiveness to Ca^{2+} [16, 20] that can be attributed to the increase in phosphorylation of the contractile proteins here described, could become more important as a mechanism mediating the relaxant action of β-agents. A decrease in myofilament Ca^{2+} sensitivity i.e. a faster dissociation of Ca^{2+} from Tn C- may account by itself for the increase in the rate of tension fall observed. However additional mechanisms may also play a role. In this context, it is likely that a cAMP-mediated phosphorylation of the Na^{+}-Ca^{2+} exchanger [35] or the SL Ca^{2+}-pump [36] could be contributing to the relaxant effect

Fig. 5. Effects of phenylephrine on different parameters of the isometric twitch. Experiments were carried out at 22°C. Under these experimental conditions, the positive inotropic effect of phenylephrine (increase of DT and $+\dot{T}$) was partially abolished by 10^{-6} M propranolol while the relaxant effect (decrease of the ratio $+\dot{T}/-\dot{T}$ and $t_{1/2}$) became negative lusitropic (increase of the ratio $+\dot{T}/-\dot{T}$ and $t_{1/2}$). 10^{-6} M prazosin completely abolished the remanent positive inotropic and negative lusitropic effects. O: phenylephrine, ●: phenylephrine plus propranolol, Δ: phenylephrine plus propranolol plus prazosin.

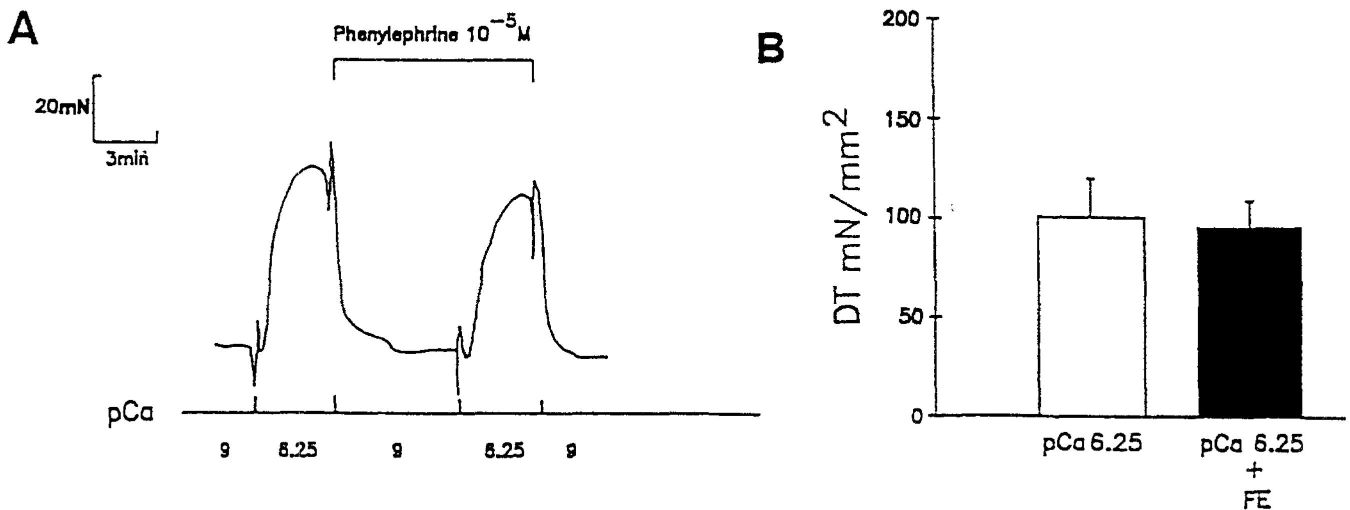

Fig. 6. Evaluation of the Ca^{2+} responsiveness of the contractile system. Phenylephrine did not change the myofibrillar sensitivity to Ca^{2+} studied in hyperpermeable trabeculae from toad heart. A: Records of a typical experiment showing contraction (pCa 6.25) and relaxation (pCa 9) cycles with or without 10^{-5} M phenylephrine. B: Overall results of developed tension (DT) obtained at pCa 6.25 in the presence or absence of phenylephrine. Experiments were carried out at 22°C. Bars represent means ± SE. FE: phenylephrine.

of β-stimulation in amphibian heart by increasing the rate of Ca^{2+} removal from the cytosol.

α-adrenergic stimulation

The fact that at 30°C the positive inotropic effect of phenylephrine was associated with a positive lusitropic action and that both effects were decreased to non significant levels by propranolol indicate that this response was mainly obtained through β-adrenoceptors stimulation. At 22°C phenylephrine elicits a small propranolol-resistant, prazosin-sensitive positive inotropic effect which is now associated with a negative lusitropic action. Although rather small, these effects are consistent and significant.

Previous studies performed in amphibian ventricle were able to show a positive inotropic effect of adrenaline at low temperature which could be blocked by 26.5 uM of phentolamine [23]. Since this concentration and the concentrations of the α-adrenoceptors blocking drugs used by others [25] were very high, it has been suggested that the blocking drugs acted by their myocardial depressant property [37]. In the present experiments the positive inotropic effect of phenylephrine was blocked by a relatively low concentration of prazosin (1 μM). This is in agreement with the results obtained by Niedergerke and Page [24] who were able to block part of the effect of adrenaline in frog single atrial trabeculae with 1 μM phentolamine. Besides, the concentration of the antagonist used in the present experiments, had no detectable effect on any of the mechanical parameters studied. Thus, the lack of effect of phenylephrine in the presence of prazosin can not be attributed to a depressant effect of the blocking agent.

The mechanism of the positive inotropic effect of phenylephrine is still a matter of controversy in mammalian heart. It has been suggested that an increase in myofibrillar sensitivity to Ca^{2+} may play an important role [5]. This mechanism may also explain the negative lusitropic effect of the drug [4]. Although the positive inotropic and negative lusitropic effect of phenylephrine in the toad ventricle was rather small, attempts were made to detect a significant change in myofibrillar sensitivity to Ca^{2+}, after phenylephrine pretreatment, that could explain the mechanical effects observed. The results of these experiments were negative. This is so even though the experiments were performed at 22°C, the temperature at which the positive inotropic and negative lusitropic effects were observed. This may indicate either that an increase in myofibrillar sensitivity to Ca^{2+} was not involved in the positive inotropic effect of α-adrenergic stimulation or that the increase in Ca^{2+} myofibrillar sensitivity occurred through an intracellular alkalinization via activation of Na^+/H^+ exchanger by protein kinase C [7]. If this was the case, the increase in Ca^{2+} myofilament sensitivity would not be detected in a chemically skinned perfused at constant pH.

Other mechanisms, like a release of Ca^{2+} by the SR [38] or a prolongation of the action potential, probably involved in the positive inotropic and negative lusitropic effects of α-adrenergic stimulation in mammalian heart [39, 40], may also play a role in the amphibian ventricle. A prolonged action potential will increase Ca^{2+} influx through voltage-dependent Ca^{2+} channels [40]. Recently Fedida and Bouchard [39] provided evidence that the increase in contractile force produced by α-agonists can be observed only when the action potential duration was increased. A prolongation of the action potential may also contribute to a negative lusitropic effect [41]. This mechanism may be important in amphibian heart which contractile tension is mainly dependent on the extracellular Ca^{2+} [42]. An increase in Ca^{2+} influx following phenylephrine stimulation have indeed been observed in frog ventricular cells [43]. Further investigation is needed to clarify this point. In any case, our results have shown that α-adrenergic positive inotropic effect is associated with a small but significant negative lusitropic effect in toad ventricle. However, these effects can only be detected at low temperature and are completely masked by a predominant β-adrenergic effect at 30°C.

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