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Physiological antagonism between ventricular β_1-adrenoceptors and α_1-adrenoceptors but no evidence for β_2- and β_3-adrenoceptor function in murine heart

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1 Murine left atrium lacks inotropic β_2-adrenoceptor function. We investigated whether β_2-adrenoceptors are involved in the cardiostimulant effects of (−)-adrenaline on spontaneously beating right atria and paced right ventricular myocardium of C57BL6 mice. We also studied a negative inotropic effect of (−)-adrenaline.

2 Sinoatrial tachycardia, evoked by (−)-adrenaline was resistant to blockade by β_2-selective ICI 118,551 (50 nM) but antagonized by β_1-selective CGP 20712A (300 nM). This pattern was unaffected by pretreatment with pertussis toxin (PTX, 600 μg kg^{-1} i.p. 24 h) which reversed carbachol-evoked bradycardia to tachycardia.

3 Increases of ventricular force by (−)-adrenaline and (−)-noradrenaline were not blocked by ICI 118,551 but antagonized by CGP 20712A.

4 Under blockade of β-adrenoceptors, (−)-adrenaline and (−)-noradrenaline depressed ventricular force ($-\log IC_{50}M = 7.7$ and 6.9). The cardiodepressant effects of (−)-adrenaline were antagonized by phentolamine (1 μM) and prazosin (1 μM) but not by (−)-bupranolol (1 μM). Prazosin potentiated the positive inotropic effects of (−)-adrenaline (in the absence of β-blockers) from $-\log EC_{50}M = 6.2-6.8$.

5 PTX-treatment reduced carbachol-evoked depression of ventricular force in the presence of high catecholamine concentrations. Inhibition of ventricular function of G_i protein was verified by 82% reduction of *in vitro* ADP-ribosylation. PTX-treatment tended to increase the positive inotropic potency of (−)-adrenaline under all conditions investigated, including the presence of ICI 118,551.

6 (−)-Adrenaline causes murine cardiostimulation through β_1-adrenoceptors but not through β_2-adrenoceptors. The negative inotropic effects of (−)-adrenaline are mediated through ventricular α_1-adrenoceptors but not through β_3-adrenoceptors. Both G_i protein and α_1-adrenoceptors restrain (−)-adrenaline-evoked increases in right ventricular force mediated through β_1-adrenoceptors.
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Keywords: Mouse heart; α_1-adrenoceptors; β_1-adrenoceptors; functional antagonism; pertussis toxin; lack of β_2-adrenoceptor function

Abbreviations: CGP 20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((methyl-4-trifluormethyl) 1 H imidazole-2-yl)- phenoxy)propyl) amino)ethoxy)-benzamide monomethane sulphonate); ICI 118,551, (erythro-DL-1 (7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol); L-NMMA, N^G-monomethyl-L-arginine monoacetate; PTX, pertussis toxin; SR59230A (3-(2-ethylphenoxy)-1-[(1**S**)-1,2,3,4-tetrahydronaphth-1-ylamino]-2**S**-2-propanol oxalate).

Introduction

Since the first report by Carlsson *et al*. (1972) for feline sinoatrial node, co-functioning cardiac β_1- and β_2-adrenoceptors have been verified in a variety of mammals. (−)-Adrenaline increases heart rate and force usually more through β_1-adrenoceptors than through β_2-adrenoceptors. There are not only quantitative but also qualitative differences of β_2-adrenoceptor function between species. For example, the increase of sinoatrial rate through β_2-adrenoceptors with adrenaline is small in the rat (Kaumann, 1986), more important in the ferret (Lowe *et al*., 2002) and marked in the cat (Lemoine & Kaumann, 1991). Ventricular contractions can also be enhanced through β_2-adrenoceptors but in several species the β_1-adrenoceptor-mediated shortening of contraction and hastening of relaxation is not observed with β_2-adrenoceptor activation. This qualitative difference of positive inotropic effects between β_1- and β_2-adrenoceptors was first reported in feline ventricle (Lemoine & Kaumann, 1991) and later confirmed for sheep ventricle (Borea *et al*., 1992) and rat ventricular myocytes (Xiao & Lakatta, 1995). Kutznetsov *et al*. (1995) observed β_2-adrenoceptor-mediated increased contractility and Ca^{2+} transients in ventricular myocytes from neonatal rats but not from adult rats.

In man, both β_1- and β_2-adrenoceptors share the mediation of cardiostimulant effects of physiological catecholamines and the relative role of β_2-adrenoceptors is usually more

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important than in other mammals. (−)-Adrenaline can be a more potent inotropic agonist through β_2-adrenoceptors than noradrenaline through β_1-adrenoceptors, particularly in atria from patients chronically treated with β-blockers (Kaumann *et al*., 1989; Hall *et al*., 1990). Positive inotropic effects of adrenaline, mediated through β_2-adrenoceptors, have also been reported for human non-failing ventricle (Kaumann & Lemoine, 1987), ventricular myocytes (Del Monte *et al*., 1993) and trabeculae from patients in heart failure (Kaumann *et al*., 1999). In ventricular myocardium from failing hearts (Kaumann *et al*., 1999) but also from non-failing infant hearts (Molenaar *et al*., 2000) the positive intropic effects of adrenaline, mediated through β_2-adrenoceptors, are as potent and nearly as efficacious as the effects of (−)-noradrenaline, mediated through β_1-adrenoceptors. The increases in contractile force mediated through both human cardiac β_1- and β_2-adrenoceptors are accompanied by positive lusitropy and phosphorylation of the proteins involved in hastened relaxation, phospholamban and troponin I, in atrium (Kaumann *et al*., 1996) and ventricle (Kaumann *et al*., 1999; Molenaar *et al*., 2000). Therefore, β_1- and β_2-adrenoceptor-mediated contractile and lusitropic effects are consistent with coupling of the receptors to G_s protein.

The general consensus that cardiac β_1-adrenoceptors couple to a G_s protein/cyclic AMP pathway does not apply to β_2-adrenoceptors for a variety of species. Lemoine & Kaumann (1991) suggested that the β_2-adrenoceptor-mediated inotropic responses to (−)-adrenaline in feline ventricle were unrelated to cyclic AMP, and similar observations were made in canine ventricular myocytes (Altschuld *et al*., 1995). It has been subsequently proposed that β_2-adrenoceptors couple concurrently to G_s and G_i protein in ventricular myocytes from adult rats (Xiao & Lakatta, 1995; but see Laflamme & Becker, 1998) and mice (Xiao *et al*., 1999), and that G_s protein-mediated cardiostimulation in mouse becomes only apparent after inactivation of G_i with PTX pre-treatment. However, two lines of evidence suggest that after PTX pre-treatment β_2-adrenoceptor-mediated responses in ventricular cardiomyocytes from mice are independent of increases in cyclic AMP and therefore not mediated through transduction by G_s protein. Sabri *et al*. (2000) did not detect significant increases in cyclic AMP in murine neonatal cardiomyocytes with the β_2-selective agonist zinterol at concentrations that increased contractility, regardless of whether the myocytes were treated or untreated with PTX. Most recently, Devic *et al*. (2001) found a small increase of spontaneous beating rate of neonatal ventricular cardiomyocytes from β_1-adrenoceptor knockout mice (β_1KO) mediated through β_2-adrenoceptors that was unaffected by an inhibitor of cyclic AMP-dependent protein kinase. The small β_2-adrenoceptor-mediated increase in beating rate was augmented after PTX pre-treatment while a small increase in cyclic AMP levels was not (Devic *et al*., 2001). Therefore, β_2-adrenoceptors of neonatal ventricular cardiomyocytes of mice appear to couple to biochemical pathways unrelated to G_s protein stimulation, even after inactivation of the G_i protein with PTX. In contrast to the evidence in murine neonatal cardiomyocytes from β_1KO mice which show some β_2-adrenoceptor-mediated responses to (−)-isoprenaline, (−)-isoprenaline does not enhance heart rate in adult β_1KO mice (Rohrer *et al*., 1996), suggesting that adult mice lack sinoatrial β_2-adrenoceptor function. Furthermore, in left atrium of adult wild-type mice, (−)-adrenaline increases contractile force only through β_1-adrenoceptors and PTX-treatment fails to uncover any participation of β_2-adrenoceptors (Oostendorp & Kaumann, 2000). We have now examined whether (−)-adrenaline can produce sinoatrial tachycardia and increase ventricular contractility through β_2-adrenoceptors of adult wild-type mice.

We previously estimated that the ventricular β-adrenoceptor population of wild-type mice consists of 71% β_1-adrenoceptors and 29% β_2-adrenoceptors (Heubach *et al*., 1999), but there is no evidence whether this β_2-adrenoceptor population can mediate enhanced contractility. We investigated the cardiostimulant effects of (−)-adrenaline on spontaneously beating right atria and paced right ventricular free wall of wild-type C57BL6 mice under conditions of selective activation of β_1- or β_2-adrenoceptors. Effects of (−)-noradrenaline were studied for comparison. To eliminate a possible G_i-induced prevention of cardiostimulation, we also studied the responses to (−)-adrenaline after treatment of mice with PTX. To assess the effectiveness of G_i inactivation by PTX, we tested for carbachol-evoked cardiodepression, mediated through muscarinic M_2 receptors coupled to G_i/G_o protein (Tucek *et al*., 1987; Fleming *et al*., 1988; Adamson *et al*., 1993). G_i inactivation by PTX was also verified by *in vitro* ADP-ribosylation (Grimm *et al*., 1998).

Methods

Isolated cardiac tissues

The experiments complied with the regulations of the German Home Office regarding care and use of laboratory animals. Male C57BL6 mice of 3–5 months of age were killed by dislocation of the neck. The hearts were immediately dissected and placed in oxygenated, modified Tyrode solution at room temperature containing (mM): NaCl 126.7, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 22.0, NaH_2PO_4 0.42, EDTA 0.04, ascorbic acid 0.2, glucose 5.0. The pH of the solution was maintained at 7.4 by passing a mixture of 5% CO_2 and 95% O_2 into the bath. Right atria and the free wall of the right ventricle were rapidly dissected. The remainder of the ventricles were snap-frozen and stored in liquid nitrogen for *in vitro* ADP-ribosylation. After dissection, pairs of tissues were mounted and attached to Swema 4.45 strain gauge transducers in an apparatus (Blinks, 1965) containing above solution at 37°C, supplemented with cocaine 3 μM (to block neuronal uptake of catecholamines), corticosterone 30 μM (to block extraneuronal uptake of catecholamines), and phentolamine 1 μM (to block α_1 and α_2-adrenoceptors) or prazosin 1 μM (to block α_1-adrenoceptors) or by combination of both α-adrenoceptor blockers. Some experiments with ventricular preparations were also carried out in the absence of α-adrenoceptor blocking agent.

Spontaneously beating right atria were set up to develop just enough tension to obtain stable continuous measurements of the frequency of contractions.

A single triangular strip was prepared from each right ventricle and paced to contract at 2 Hz with square-wave pulses of 5 ms duration and just over threshold currents. After determining a length–force curve, the ventricular strips were left at the length associated with maximum developed

force. A stabilization period of 2.5 h was allowed before starting the addition of agonists. The time course of basal ventricular force was investigated in six right ventricles. Basal force (mN), at 2.5, 3.5, 4.5 and 5.5 h after determination of the length–tension curve was 1.08 ± 0.08, 0.90 ± 0.08, 0.79 ± 0.08, 0.74 ± 0.08 and 0.70 ± 0.07 respectively. All agonist effect were studied during this 3 h period.

The width of two free right ventricular walls was assessed with 6–8 Azan-stained sections against a 0.37 mm diameter wire for each tissue. The width of the unstretched free wall of the right ventricle ranged from 0.4 to 0.6 mm for the thinnest and thickest portions.

Rate and force were recorded with the help of PowerLab amplifiers on a Chart for Windows, Version 4.0 recording programme (ADInstruments, Castle Hill, NSW, Australia).

Pertussis toxin pretreatment and ADP-ribosylation

Mice were injected with 600 μg kg^{-1} i.p. PTX or the same volume of 0.9% NaCl (12 μl g^{-1} mouse). Twenty-four hours later paired right atria and paired right ventricular strips from a PTX-untreated and PTX-treated mouse were set up into the same organ bath whenever possible.

Due to inconsistent activity in commercial batches of PTX, we report only data from PTX-treated mice in which carbachol did not produce negative inotropic (right ventricles) or negative chronotropic effects (right atria). PTX-catalysed ADP-ribosylation was performed in crude ventricular homogenates (30 μg protein) of control and PTX-treated mice in the presence of ^{32}P-NAD as described previously (Vandecasteele *et al*., 1999). After ADP-phosphorylation, proteins were subjected to SDS–PAGE (9% acrylamide, 37.5 : 1, 6 M urea), subsequent Western blotting and autoradiography/phosphoimaging of dried membranes. For quantification, each gel contained a dilution of standard homogenate protein to verify linearity of the assay. Each value was expressed as per cent of the mean of all (five/six) control samples on one blot. Normalization for protein loading was performed by immunodetection of calsequestrin using a polyclonal rabbit antibody (Dianova, Hamburg, Germany) and subsequent enhanced chemiluminescence detection (Amersham-Pharmacia, Freiburg, Germany).

Experimental design

CGP 20712A (Dooley *et al*., 1986) and ICI 118,551 (Bilski *et al*., 1983) were used as tools to selectively block β_1- and β_2-adrenoceptors, respectively. Antagonists were incubated for 90 min before an agonist was administered.

Spontaneously beating right atria To investigate whether CGP 20712A and ICI 118,551 modified sinoatrial rate, cumulative concentration–effect curves were carried out between 1 nM and 10 μM. Twenty minutes were allowed for each concentration to equilibrate. Due to the long time course, the beating rate of atria not exposed to drugs was also measured during the entire length of the experiment (Figure 1).

Concentration–effect curves to (−)-adrenaline and (−)-noradrenaline were determined in the absence or presence of β_1-selective CGP 20712A (300 nM) or β_2-selective ICI 118,551 (50 nM). The experiments were terminated by saturating the β-adrenoceptors with (−)-isoprenaline (200 μM). Only a single curve was determined on each atrium.

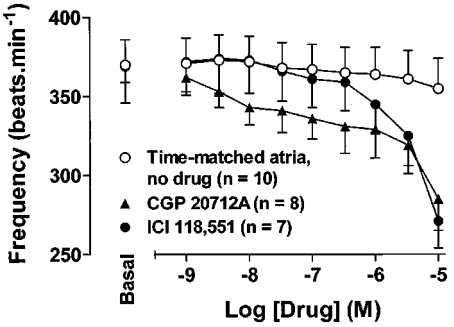

Figure 1 Negative chronotropic effects of CGP 20712A and ICI 118,551 on spontaneously beating right atria.

Carbachol-induced bradycardia is assumed to be mediated through G_i protein-coupled M_2 receptors (Caulfield & Birdsall, 1998). PTX-pretreatment inactivates G_i function and would be expected to abolish carbachol-induced bradycardia. To assess the effectiveness of PTX we compared cumulative concentration–effect curves for carbachol (0.2–60 μM) on right atria, obtained from mice not treated and treated with PTX. Atrial beating rate was allowed to stabilize for 3 h before the curve for carbachol was begun.

Right ventricle The protocol of Oostendorp & Kaumann (2000) was usually applied for ventricular strips. Cumulative concentration–effect curves for (−)-adrenaline and (−)-noradrenaline were determined in the absence and presence of CGP 20712A (300 nM) or ICI 118,551 (50 nM) or combination with the two antagonists. (−)-Isoprenaline (200 μM) was administered in the presence of the highest used concentration of (−)-noradrenaline or (−)-adrenaline, and a cumulative concentration–effect curve to carbachol (0.2–60 μM) was carried out in the presence of the catecholamines. The experiments were terminated by raising the $CaCl_2$ concentration to 10 mM. The same protocol was usually used with ventricles obtained from PTX-treated mice.

Statistics $-\text{LogEC}_{50}$ and $-\text{logIC}_{50}$ values for catecholamines were estimated from fitting curves to a Hill function with variable slope to concentration–effect curves from individual experiments. The data are expressed as mean ± s.e.mean of n = number of mice. Significance between differences were assessed by using the alternate t-test (Welch-test), the paired Student's t-test or with ANOVA followed by Bonferroni or Dunnett *post-hoc* test at $P < 0.05$ using Instat software.

Drugs ^{32}P-NAD (1000 Ci/mmole) was from Amersham-Pharmacia (Freiburg, Germany). PTX was from Sigma (Deisenhofen, Germany) for *in vivo* treatment of mice and from Calbiochem (Bad Soden, Germany) for *in vitro* ADP-ribosylation. (−)-Adrenaline hydrochloride, (−)-noradrenaline hydrochloride, (−)-isoprenaline hydrochloride, carbachol, corticosterone, CGP 20712A, atropine, (−)-propranolol, prazosin and phentolamine were from Sigma.

Cocaine was purchased from Merck (Darmstadt, Germany). ICI 118,551 from Tocris (Bristol, UK) and L-NMMA from Alexis (Grünberg, Germany). (−)-Bupranolol was a gift from Schwarz Pharma (Monheim, Rheinland, Germany). SR59230A was a gift of Dr L. Manara (Sanofi, Milan, Italy).

Results

Negative chronotropic effects of CGP 20712A and ICI 118,551

CGP 20712A reduced sinoatrial rate in a concentration-dependent and biphasic manner (Figure 1). A high potency component was observed between 1–1000 nM that tended to be maximal between 300 and 1000 nM with $-\log IC_{50}$, M = 8.83 ± 0.22 and a decrease of 42 ± 14 beats min^{-1} at 1000 nM ($P = 0.079$, $n = 8$). The low potency component was observed between 3 and 10 μM and produced a bradycardia of 86 ± 17 beats min^{-1} at 10 μM ($P < 0.005$). ICI 118,551 reduced sinoatrial rate between 1 and 10 μM (Figure 1), as observed previously in guinea-pig left atrium (Bilski *et al*., 1983).

The slight bradycardia produced by CGP 20712A at low concentrations could possibly be due either to blockade of the effects of small amounts of noradrenaline released from the nerve endings or to inverse agonism at β_1-adrenoceptors. The $-\log IC_{50}$M of 8.8 on right atria is consistent with the affinity estimate of CGP 20712A for murine β_1-adrenoceptors (Heubach *et al*., 1999), perhaps making more likely the first alternative.

Carbachol-evoked tachycardia after PTX treatment

Carbachol caused bradycardia in right atria from non-PTX-treated mice. In atria from PTX-treated mice carbachol produced tachycardia that was reversed by atropine (1 μM) (Figure 2). The carbachol-evoked tachycardia in right atria from PTX-treated mice was resistant to antagonism by (−)-propranolol (200 nM). The tachycardia produced by 60 μM carbachol amounted to 86 ± 26 ($n = 7$) and 67 ± 16 beats/min ($n = 4$) in the absence and presence of (−)-propranolol respectively ($P = 0.55$).

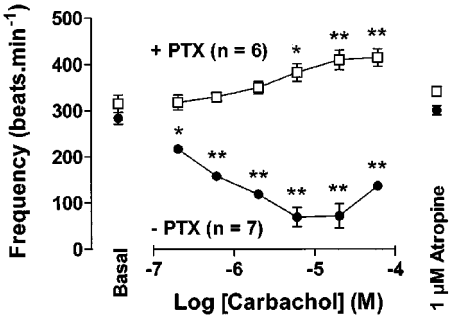

Figure 2 Prevention by PTX of the carbachol-evoked bradycardia and reversal to tachycardia in spontaneously beating right atria. Atropine, administered after an equilibrium effect with 60 μM carbachol, reversed both bradycardia (−PTX) and tachycardia (+PTX) to basal levels. Significance between basals and carbachol effects: $^*P < 0.05$, $^{**}P < 0.01$.

CGP 20712A but not ICI 118,551 blocks catecholamine-evoked tachycardia

(−)-Adrenaline and (−)-noradrenaline elicited sinoatrial tachycardia with similar potency (Figure 3a,b; Table 1). CGP 20712A (300 nM) produced significant bradycardia and shifted the concentration-effect curves of (−)-adrenaline and (−)-noradrenaline by over two log units to higher catecholamine concentrations (Figure 3a,b; Table 1). The positive chronotropic effects of both catecholamines were resistant to blockade by ICI 118,551 (50 nM), either in the absence or presence of CGP 20712A (Figure 3a,b; Table 1).

PTX does not influence catecholamine effects on right atria

PTX did not modify the chronotropic potencies of (−)-adrenaline and (−)-noradrenaline (Figure 3c,d; Table 1). The CGP 20712A-induced bradycardia and shift of the chronotropic concentration-effect curves for (−)-adrenaline and (−)-noradrenaline were not significantly different between right atria from PTX-untreated and PTX-treated mice (Figure 3; Table 1). The lack of PTX effect on the positive chronotropic responses to the catecholamines agrees with similar observations in the rat (Grimm *et al*., 1998). ICI 118,551 did not block significantly the positive chronotropic effects of (−)-adrenaline and (−)-noradrenaline in right atria from PTX-pretreated mice (Figure 3c,d; Table 1). For unknown reasons, ICI 118,551 in the presence of CGP 20712A slightly sensitized atria from PTX-treated mice to (−)-adrenaline (Table 1).

Ventricular effects of catecholamines in the presence of phentolamine

The positive inotropic effects of (−)-adrenaline (Figures 4 and 5) and (−)-noradrenaline (Figure 6) in right ventricular strips were not significantly changed by ICI 118,551 (50 nM) (Figures 4–6, Table 2). Under CGP 20712A (300 nM) both catecholamines caused negative inotropic effects at low concentrations, which were unaffected by ICI 118,551, and positive inotropic effects at high concentrations (Figures 4–6, Table 2). ICI 118,551 in the presence of CGP 20712A did not antagonize the positive inotropic effects of (−)-adrenaline (Figure 5, Table 2) or (−)-noradrenaline (Figure 6, Table 2).

Ventricular effects of PTX treatment in the presence of phentolamine

The negative inotropic effects of carbachol (20 μM) in the presence of both (−)-adrenaline and (−)-isoprenaline were highly significant in ($P < 0.001$, $n = 24$) but not after PTX treatment ($P = 0.237$, $n = 22$) (Figures 4 and 5). Similarly, the negative inotropic effects of carbachol (20 μM) in the presence of both (−)-noradrenaline and (−)-isoprenaline were highly significant ($P < 0.001$, $n = 27$) but not after PTX treatment ($P = 0.148$, $n = 15$) (Figure 6).

PTX treatment of the mice tended to enhance the inotropic potency of (−)-adrenaline and (−)-noradrenaline (Table 2, Figures 4–6). ICI 118,551 (50 nM) did not antagonize significantly the effects of (−)-adrenaline and (−)-noradrenaline in the absence and presence of CGP 20712A (300 nM)

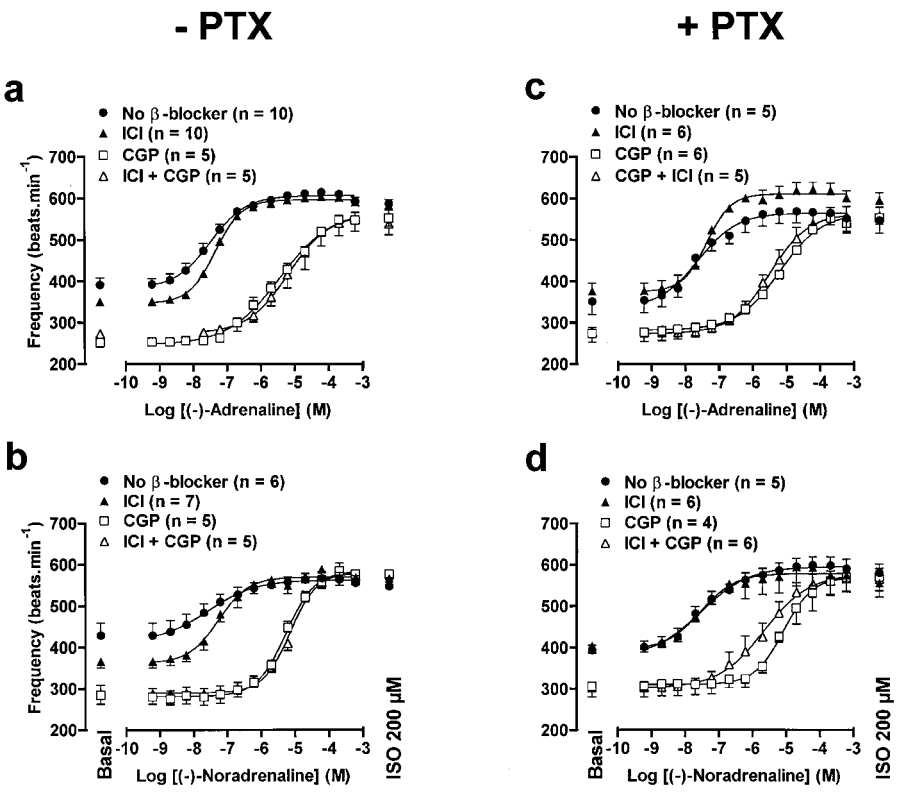

Figure 3 Blockade by CGP 20712A (300 nM – CGP) but not by ICI 118,551 (50 nM – ICI) of the positive chronotropic effects of (−)-adrenaline (a,c) and (−)-noradrenaline (b,d). No significant effects of PTX treatment (c,d). ISO = (−)-isoprenaline (200 μM). No significant differences between the maximal chronotropic effects were detected in each panel.

Table 1 Chronotropic potencies of (−)-adrenaline and (−)-noradrenaline in murine right atria

	(−)-Adrenaline				*(−)-Noradrenaline*			
	−PTX		*+PTX*		*−PTX*		*+PTX*	
	n	$-log\ EC_{50}$	*n*	$-log\ EC_{50}$	*n*	$-log\ EC_{50}$	*n*	$-log\ EC_{50}$
No β-blocker	10	7.46±0.10	5	7.60±0.05	6	7.45±0.20	5	7.47±0.18
ICI 118,551 (50 nM)	10	7.32±0.05	6	7.33±0.05	7	7.22±0.14	6	7.52±0.13
CGP 20712A (300 nM)	5	5.42±0.22	6	5.13±0.08	5	5.28±0.05	4	4.99±0.14
CGP 20712A (300 nM) +ICI 118,551 (50 nM)	5	5.10±0.18	5	5.48±0.07**	5	5.17±0.07	6	5.81±0.34

**$P<0.01$ CGP 20712A + ICI 118,551 vs CGP 20712A (ANOVA). The effects of CGP 20712A were highly significant ($P<0.001$, ANOVA).

(Figures 4–6, Table 2). PTX treatment increased significantly the positive inotropic potency of (−)-adrenaline in the presence of ICI 118,551 or CGP 20712A (Table 2). PTX treatment also enhanced significantly the positive inotropic potency of (−)-noradrenaline in the presence of CGP 20712A or in the presence of both ICI 118,551 and CGP 20712A (Table 2).

(−)-Bupranolol does not block the negative inotropic effects of (−)-adrenaline

The negative inotropic effects of (−)-adrenaline and (−)-noradrenaline in the presence of CGP 20712A could be due to activation of β_3-adrenoceptors, as reported for human ventricle (Gauthier *et al*., 1996) and attributed to release of nitric oxide (NO) (Gauthier *et al*., 1998). However, the negative inotropic effects of (−)-adrenaline in the presence of CGP 20712A (300 nM) and ICI 118,551 (50 nM) were not antagonized by (−)-bupranolol at a concentration, 1 μM, that blocks β_3-adrenoceptors (Arch & Kaumann, 1993) (Figure 7b). Furthermore, 1 μM of the β_3-adrenoceptor-selective antagonist SR 59230A (Manara *et al*., 1995) did not block the negative inotropic effects of (−)-adrenaline ($n=2$ ventricles, results not shown), excluding an involvement of β_3-adrenoceptors. L-NMMA (0.5 mM) failed to affect the negative inotropic effects of (−)-adrenaline ($n=4$, experi-

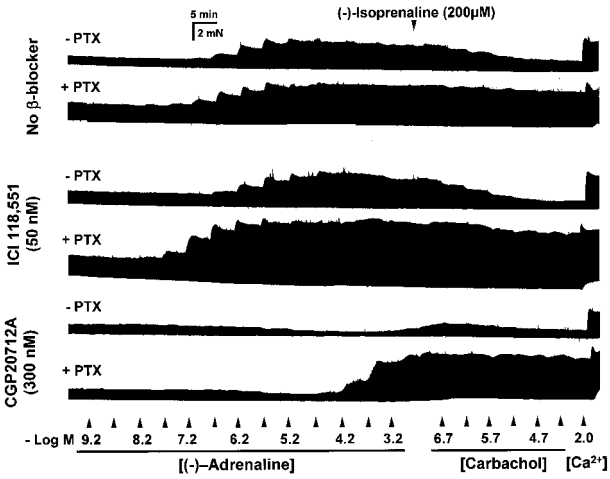

Figure 4 Lack of blockade of the positive inotropic effects of (−)-adrenaline by ICI 118,551 in right ventricular wall from PTX-untreated and PTX-treated mice. Reduction of the negative inotropic effects of carbachol by PTX treatment. Negative inotropic effects of (−)-adrenaline in the presence of CGP 20712A. Representative tracings of experiments carried out in the presence of phentolamine (1 μM). Notice that the positive intropic effects of (−)-adrenaline tend to be enhanced and the appearance of Ca^{2+}-contractures in preparations from PTX-treated mice.

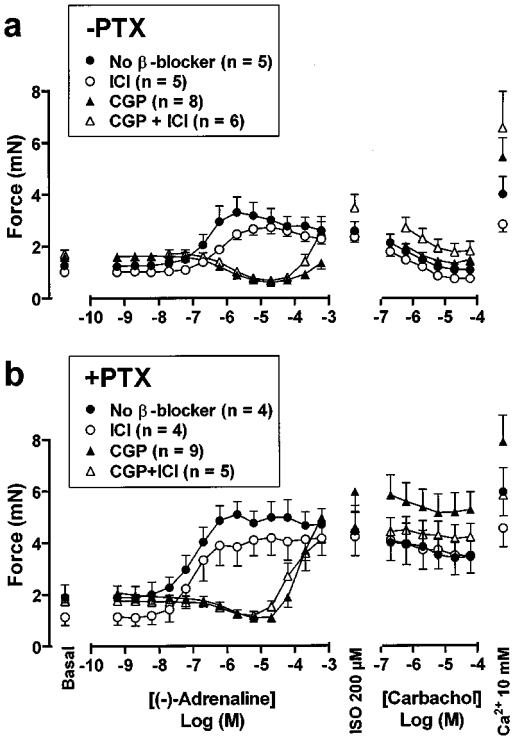

Figure 5 Blockade by CGP 20712A (300 nM – CGP) but not by ICI 118,551 (50 nM – ICI) of the positive inotropic effects of (−)-adrenaline in ventricular strips from PTX-untreated (−PTX) (a) and PTX-treated (+PTX) (b) mice. Attenuation of the negative inotropic effects of carbachol by PTX. ISO = (−)-isoprenaline. Experiments carried out in the presence of phentolamine (1 μM). Notice that CGP 20712A unconceals negative inotropic effects of (−)-adrenaline that are resistant to both blockade by ICI 118,551 and PTX treatment. PTX treatment enhanced the maximum effects of (−)-adrenaline effects in CGP20712A groups ($P<0.01$, ANOVA) but not significantly in the other groups.

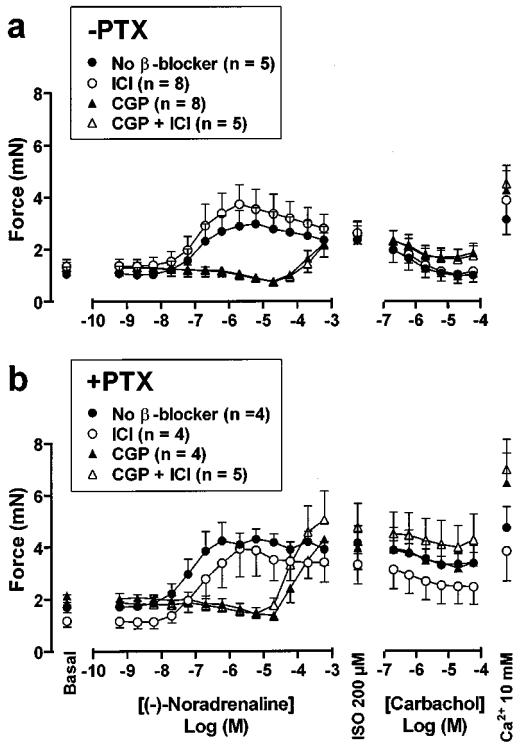

Figure 6 Blockade by CGP 20712A (300 nM – CGP) but not by ICI 118,551 (50 nM – ICI) of the positive inotropic effects of (−)-noradrenaline in ventricular strips from PTX-untreated (a) and PTX-injected (b) mice. Other details as in Figure 5. Experiments carried out in the presence of phentolamine (1 μM). Notice that CGP 20712A uncovers negative inotropic effects of (−)-noradrenaline. PTX treatment did not significantly change the maximum effects of (−)-noradrenaline in the four groups (ANOVA).

ments not shown), excluding an involvement of NO. (−)-Bupranolol, in the presence of both CGP 20712A and ICI 118,551, reduced the appearance of positive inotropic effects of high (−)-adrenaline concentrations, compared to the combination of CGP 20712A and ICI 118,551 alone. To better study the negative inotropic effects of the catecholamines we performed experiments in the presence of the 3 β-blockers (Figure 7a,c).

Comparison of the negative inotropic effects of (−)-adrenaline and (−)-noradrenaline

(−)-Adrenaline ($-\log IC_{50}M = 7.70 \pm 0.02$, $n=5$) was a significantly more potent negative inotropic agonist than (−)-noradrenaline ($-\log IC_{50}M = 6.93 \pm 0.07$, $n=5$, $P<0.0005$, Figure 7a,b).

Antagonism of the negative inotropic effects of (−)-adrenaline by phentolamine and prazosin

It has been suggested that prazosin-sensitive α-adrenoceptors mediate negative inotropic effects of phenylephrine in mouse ventricle (Tanaka *et al*., 1995). To establish whether or not the ventricular negative inotropic effects of (−)-adrenaline are mediated through α-adrenoceptors, we carried out experiments in the presence of CGP 20712A, ICI 118,551 and (−)-bupranolol in the absence and presence of prazosin

Table 2 Positive inotropic potencies of (−)-adrenaline and (−)-noradrenaline the presence of phentolamine (1 μM) in murine right ventricle

	(−)-Adrenaline				(−)-Noradrenaline			
	−PTX		+PTX		−PTX		+PTX	
	n	$-log\ EC_{50}$	*n*	$-log\ EC_{50}$	*n*	$-log\ EC_{50}$	*n*	$-log\ EC_{50}$
No β-blocker	5	6.59±0.08	4	6.95±0.15	5	6.86±0.09	4	7.04±0.25
ICI 118,551 (50 nM)	5	6.33±0.09	4	6.98±0.15*	8	6.73±0.14	4	6.79±0.15
CGP 20712A (300 nM)	8	3.08±0.05	9	3.66±0.08***	8	3.60±0.07	4	4.01±0.11*
CGP 20712A (300 nM) +ICI 118,551 (50 nM)	6	3.23±0.14	5	4.09±0.18**	5	3.44±0.09	5	4.33±0.10***

*$P<0.05$, **$P<0.01$, ***$P<0.001$ −PTX vs +PTX (Alternate *t*-test). The effects of CGP 20712A were highly significant ($P<0.001$, ANOVA).

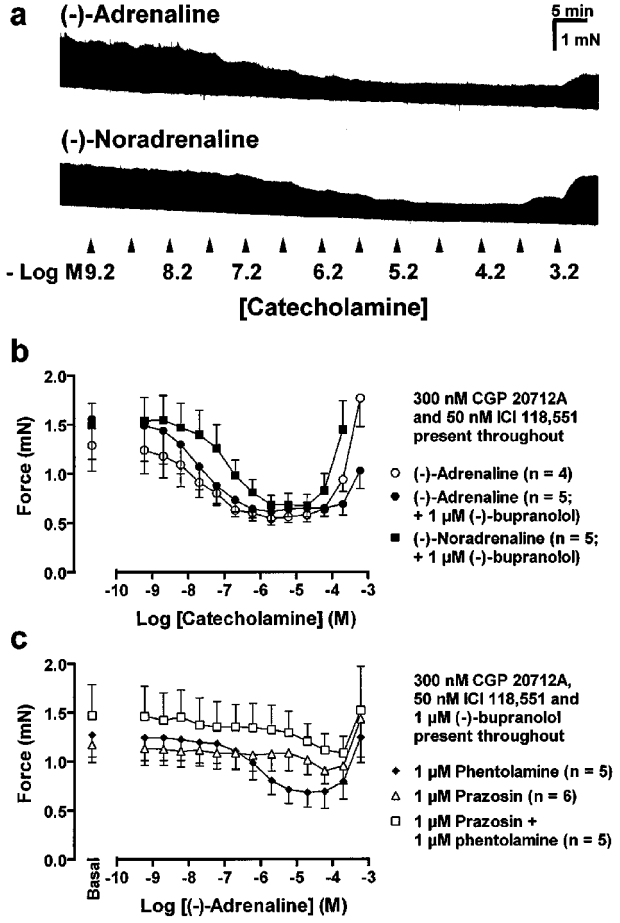

Figure 7 Comparison of the negative inotropic effects of (−)-adrenaline and (−)-noradrenaline in the presence of CGP 20712A (300 nM), ICI 118,551 (50 nM) and (−)-bupranolol (1 μM) (a,c). Tracings in (a) are representative experiments. Lack of blockade by (−)-bupranolol of the effects of (−)-adrenaline (b). Antagonism of the negative inotropic effects of (−)-adrenaline by phentolamine and prazosin, administered separately and in combination (c).

(1 μM) or phentolamine (1 μM) and combination of prazosin plus phentolamine. Phentolamine induced a surmountable shift of the concentration-effect curve for the negative inotropic effects of (−)-adrenaline by nearly 2 log units while prazosin caused a partially insurmountable shift by around 3 log units (Figure 7c). The combination of prazosin and phentolamine did not produce greater blockade than prazosin alone (Figure 7c).

ICI 118,551 does not antagonize the positive inotropic effects of (−)-adrenaline in the presence of prazosin

The α_1-adrenoceptor-mediated cardiodepression could oppose the manifestation of β_2-adrenoceptor-mediated cardiostimulation. However, under conditions of α_1-adrenoceptors blockade with prazosin (1 μM), ICI 118,551 (50 nM) did not change significantly the positive inotropic potency of (−)-adrenaline (Table 3). PTX treatment also failed to reveal an ICI 118,551-sensitive component of the positive inotropic effect of (−)-adrenaline in the absence or presence of CGP 20712A (300 nM) (Table 3). A representative experiment illustrating the lack of blockade by ICI 118,551 of the positive inotropic effects of (−)-adrenaline in the presence of both prazosin and CGP 20712A on ventricles from PTX-treated mice is shown in Figure 8.

Prazosin potentiates the positive inotropic effects of (−)-adrenaline and (−)-noradrenaline

To investigate whether the negative inotropic effects of (−)-adrenaline and (−)-noradrenaline reduce their corresponding positive inotropic potencies, we compared concentration-effect curves in the absence and presence of prazosin (1 μM). In the absence of antagonist, (−)-adrenaline caused biphasic inotropic responses, consisting of small decreases of contractile force at low concentration followed by increases in contractile force at higher concentration, as shown in the representative experiment of Figure 9 (top) and for several experiments in Figure 10a. In the presence of prazosin, the negative and positive inotropic effects of (−)-adrenaline were abolished and potentiated respectively (Figures 9 and 10a). The $-\log EC_{50}M$ for the positive inotropic effects of (−)-adrenaline were 6.17±0.06 ($n=8$) and 6.70±0.08 ($n=10$) ($P<0.001$) in the absence and presence of prazosin, respectively.

The inotropic potency of (−)-noradrenaline was only slightly increased by prazosin (1 μM) (Figure 10b). The $-\log EC_{50}M$ was 6.66±0.08 ($n=5$) and 6.98±0.10 ($n=6$,

Table 3 Positive inotropic potencies of (−)-adrenaline in the presence of prazosin (1 μM) in murine ventricle

	(−)-Adrenaline			
	−PTX		+PTX	
	n	$-log\ EC_{50}$	n	$-log\ EC_{50}$
No β-blocker	10	6.70±0.08	5	7.07±0.15
ICI 118,551 (50 nM)	4	6.66±0.09	5	6.90±0.11
CGP 20712A (300 nM)	4	3.17±0.08	7	3.84±0.08***
CGP 20712A (300 nM) +ICI 118,551 (50 nM)	5	3.35±0.18	5	4.17±0.20*

*$P<0.05$, ***$P<0.001$, −PTX vs PTX (Alternate t-test). The effects of CGP 20712A were highly significant ($P<0.001$, ANOVA).

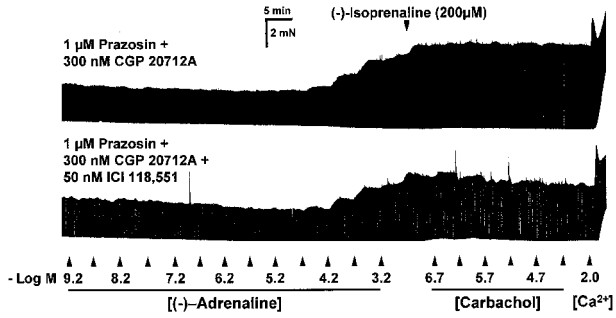

Figure 8 Lack of antagonism by ICI 118,551 (50 nM) (bottom tracing) of the positive inotropic effects of (−)-adrenaline in the presence of prazosin (1 μM). Representative preparations from two PTX-treated mice. Notice the marked Ca^{2+}-contractures.

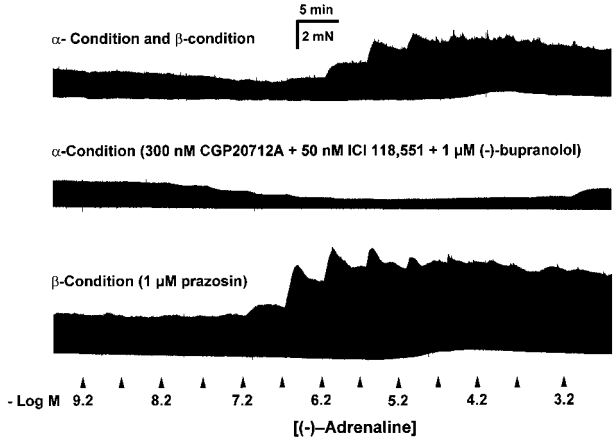

Figure 9 Positive and negative inotropic effects of (−)-adrenaline. Potentiation of the positive inotropic effects of (−)-adrenaline by prazosin (bottom tracing). Representative experiments from three ventricular preparations. Notice the biphasic inotropic effects of (−)-adrenaline in the absence of blockers (upper tracing) and that β-adrenoceptor blockade unconceals complete negative inotropic effects of (−)-adrenaline (middle tracing).

$P<0.05$) in the absence and presence of prazosin, respectively.

Potentiation of the positive inotropic effects of (−)-adrenaline by PTX treatment in the absence of adrenoceptor blockade

In the absence of both α-blockers and β-blockers, PTX treatment resulted in a significant leftward shift of the concentration–effect curve for the positive inotropic effects but disappearance of the negative inotropic effects of (−)-adrenaline (Figure 10). The $-\log EC_{50}$ (M) for (−)-adrenaline increased from 6.17±0.06 ($n=8$) in the non-PTX-treated group to 6.91±0.11 ($n=3$) ($P<0.01$) in the PTX-treated group.

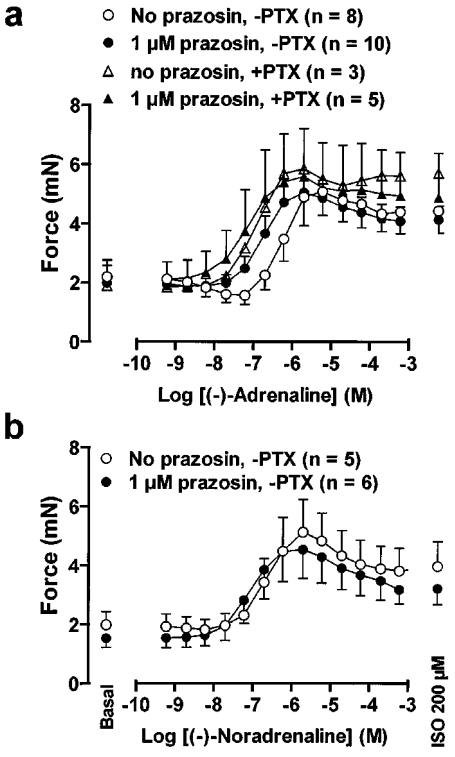

Figure 10 Marked and marginal potentiation of the positive inotropic effects of (−)-adrenaline (a) and (−)-noradrenaline (b), respectively, by prazosin. Potentiation by PTX treatment of the positive inotropic effects of (−)-adrenaline (a). The $-\log EC_{50}$ (M) for the positive inotropic effects of (−)-adrenaline in the absence of prazosin in the PTX untreated group was significantly lower from the $-\log EC_{50}$ (M) of the other three groups ($P<0.01$, ANOVA).

Increased ventricular Ca^{2+}-contractures after PTX treatment

During the exposure to 10 mM $CaCl_2$ in the presence of high catecholamine concentrations, the resting tension increased in tissues from PTX-treated mice, as illustrated in Figures 4 and

8. In two groups of ventricles that had received phentolamine, catecholamines and carbachol (but no β-adrenoceptor blocking agent), the resting tension immediately before the exposures to 10 mM $CaCl_2$ was not significantly different between ventricles from nine untreated mice (4.71 ± 0.36 mN) and eight PTX-treated mice (4.89 ± 0.47 mN). Two min after the exposure to 10 mM $CaCl_2$, the resting tension did not significantly change in the ventricles from the PTX-untreated mice (4.69 ± 0.36 mN) but increased to 5.88 ± 0.68 mN ($P < 0.01$, paired Student's t-test) in ventricles from the PTX-treated mice.

ADP-ribosylation

Successful *in vivo* ADP-ribosylation of G_i/G_o proteins was verified by *in vitro* PTX-mediated ^{32}P-ADP-ribosylation of samples from the same hearts that had been used for the preparation of atria and right ventricular free walls (Figure 11). *In vitro* PTX treatment lead to a mean reduction of ^{32}P-ADP ribose incorporation by $82 \pm 11\%$ ($n = 19$) compared to ventricles from NaCl-injected mice ($n = 16$), a value known to be maximal for this kind of experiment (Grimm *et al*., 1998).

Discussion

Our results are consistent with the sole mediation through murine β_1-adrenoceptors of the acute cardiostimulant effects of physiological catecholamines. We have failed to uncover a significant β_2-adrenoceptor function in murine sinoatrial node and right ventricle. This evidence, together with similar negative evidence in murine left atrium, suggests that acute β_2-adrenoceptor modulation of murine heart rate and force is negligible in the cardiac regions studied, at least in C57BL6 mice (this work) and Balb/c mice (Oostendorp & Kaumann, 2000). A complicating finding was the negative inotropic effect of (−)-adrenaline and (−)-noradrenaline during β_1-adrenoceptor blockade, which appears to be mediated through α_1-adrenoceptors but not through β_3-adrenoceptors. We also detected sinoatrial tachycardia elicited by carbachol after PTX-treatment.

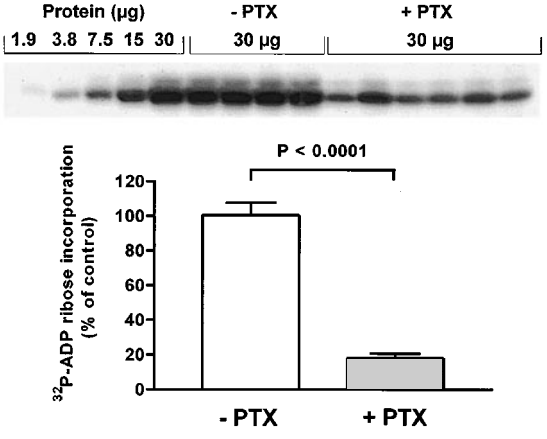

Figure 11 *In vitro* ^{32}P-ADP ribosylation in murine ventricular homogenates by PTX. Decrease of *in vitro* ^{32}P-ADP ribosylation proteins in ventricular homogenates obtained from hearts of PTX-treated mice (+PTX), demonstrating that marked ADP ribosylation had occurred *in vivo*. Representative autoradiography from a Western blot showing a standard dilution of homogenates from the ventricle of a PTX-untreated mouse (-PTX) (1.9–30 μg protein), and samples from four additional PTX-untreated mice (30 μg protein), and samples from six ventricles of PTX-treated mice (30 μg protein). Notice that the assay was in the linear range and that the *in vitro* [^{32}P-ADP]-ribose incorporation was reduced by 82% in the ventricular homogenates from PTX-treated mice (bar chart, $n = 19$), when compared to samples from PTX-untreated mice ($n = 16$).

Lack of β_2-adrenoceptor function in several regions of murine heart

We failed to find significant blockade of the sinoatrial and right ventricular effects of catecholamines by ICI 118,551 50 nM, a concentration that is equivalent to ~100 times its K_i of the β_2-adrenoceptors ($pK_i \sim 9.3$) but ~8 times smaller than its K_i for β_1-adrenoceptors ($pK_i \sim 6.4$) of the ventricle from C57BL6 mice (Heubach *et al*., 1999). ICI 118,551 also failed to block the positive chronotropic and inotropic effects of the catecholamines in the sinoatrial node and right ventricle from PTX-pretreated mice. These results and our data with CGP 20712A indicate that the positive chronotropic and inotropic effects of physiological catecholamines in the murine heart are mediated through β_1-adrenoceptors but not through β_2-adrenoceptors. For example, the data for murine sinoatrial node of Table 1 allow estimates of pK_B for CGP 20712A between 8.6 and 9.0, in line with a binding affinity estimate of CGP 20712A for ventricular β_1-adrenoceptors of C57BL6 mice ($pK_i \sim 8.1$, Heubach *et al*., 1999). Our results and conclusions are consistent with the lack of significant cardiostimulant effects of (−)-isoprenaline in mice lacking β_1-adrenoceptors (i.e. β_1-knockout – Rohrer *et al*., 1996).

Do murine cardiac β_2-adrenoceptors mediate increases in heart rate and force through a G_s protein/cyclic AMP pathway? In ventricular myocytes of wild-type mice Xiao *et al*. (1999) failed to observe effects mediated through β_2-adrenoceptors, and claimed that effects through a G_s protein/cyclic AMP-dependent pathway become apparent only after G_i inactivation with PTX. Three independent lines of evidence are, however, inconsistent with the conclusions of Xiao *et al*. (1999). (1) The positive inotropic effects of the physiological β_2-adrenoceptor agonist adrenaline in left atria from Balb/c mice were shown to be only mediated through β_1-adrenoceptors and PTX treatment does not reveal a participation of β_2-adrenoceptors (Oostendorp & Kaumann, 2000). (2) β_2-adrenoceptors are present in murine cardiomyocytes (Hilal-Dandan *et al*., 2000) but Sabri *et al*. (2000) only detected robust increases of cyclic AMP levels in ventricular myocytes from foetal ICR mice mediated through β_1-adrenoceptors but hardly a signal through β_2-adrenoceptor. Furthermore, Sabri *et al*. (2000) did not observe β_2-adrenoceptor-mediated increases in cyclic AMP levels of neonatal cardiomyocytes after PTX treatment that abolished the carbachol-induced inhibition of cyclic AMP production. Although Devic *et al*. (2001) observed that PTX enhanced the (−)-isoprenaline-induced increase of the β_2-adrenoceptor-mediated rate of beating of neonatal ventricular myocytes from β_1KO mice, the effects were not transduced by a G_s protein/cyclic AMP-dependent pathway. (3) Recent work of Heubach *et al*. (2001) has provided evidence against the β_2-adrenoceptor nature of the zinterol effects reported by Xiao *et al*. (1999). Heubach *et al*. (2001) confirmed the increase of

L-type Ca^{2+} current by zinterol after PTX treatment reported by Xiao *et al*. (1999), but found it to be blocked by CGP 20712A (300 nM) and resistant to blockade by ICI 118,551 (50 nM). However, Xiao *et al*. (1999) did not use subtype-selective antagonists to substantiate their claim. The increase of L-type Ca^{2+} channel conductance by zinterol after PTX treatment is therefore consistent with mediation through β_1-adrenoceptors but not through β_2-adrenoceptors. These three lines of evidence against a β_2-adrenoceptor function mediated through G_s protein coupling in the adult murine heart, are wholly consistent with the conclusion of our present work that only β_1-adrenoceptors but not β_2-adrenoceptors mediate the measured cardiostimulant effects of the physiological catecholamines (−)-adrenaline and (−)-noradrenaline. It is still possible, however, that β_2-adrenoceptors of murine left ventricle may mediate increases in contractility. This remains to be investigated. It is also conceivable that β_2-adrenoceptors of adult murine hearts affect cardiac function through pathways distinct from G_s protein as observed in cardiomyocytes from neonatal mice (Sabri *et al*., 2000; Devic *et al*., 2001).

The failure of (−)-adrenaline to produce positive chronotropic and inotropic effects through β_2-adrenoceptors in murine heart contrasts with the important effects mediated through β_2-adrenoceptors of human heart (Atrium: Gille *et al*., 1985; Lemoine *et al*., 1988; Hall *et al*., 1990; Kaumann *et al*., 1996; ventricle: Kaumann & Lemoine, 1987; Del Monte *et al*., 1993; Kaumann *et al*., 1999; Molenaar *et al*., 2000; sinoatrial node: Daul *et al*., 1995). Our results for sinoatrial rate and right ventricular contractility, together with those of Rohrer *et al*. (1996) and Oostendorp & Kaumann (2000), suggest that the β_1-adrenoceptor is the only functional subtype in the heart of adult wild-type mice, mediating not only the positive chronotropic and inotropic effects of physiologically occurring catecholamines but also the effects of the non-conventional partial agonist (−)-CGP12177 (Kaumann *et al*., 2001). Clearly, the adult murine heart is not a convenient model for the study of acute contractility and heart rate changes mediated by human cardiac β_2-adrenoceptors. On the other hand, because the murine heart appears to lack β_2-adrenoceptors through which adrenaline acutely enhances rate and force, it is an excellent host for expressing human β_2-adrenoceptors (Milano *et al*., 1994; Bond *et al*., 1995; Heubach *et al*., 1999), because these functions can be studied without an interfering contribution of native β_2-adrenoceptors.

A small tonic role of G_i protein in murine ventricle

G_i protein and mRNA is enhanced in heart failure (Feldman *et al*., 1988; Neumann *et al*., 1988; Böhm *et al*., 1990; Eschenhagen *et al*., 1992) and evidence that G_i blunts β-adrenoceptor responses was provided by Brown & Harding (1992), who showed that PTX restores curbed inotropic responses to (−)-isoprenaline in cardiomyocytes from patients with heart failure. The trend to enhanced inotropic responses to (−)-adrenaline in our experiments with ventricle from PTX-treated mice suggests a tonic restraining influence of G_i on β_1-adrenoceptor-mediated responses and is in line with similar conclusions from a detailed analysis in the rat (Grimm *et al*., 1998). However, this effect is only apparent to a moderate degree in ventricle and absent in sinoatrial node of both rat (Grimm *et al*., 1998) and mouse (present study), and also absent in murine left atrium (Oostendorp & Kaumann, 2000). Because the β_1-adrenoceptor appears to couple only to G_s but not to G_i protein (Xiao *et al*., 1999; Kilts *et al*., 2000), an increased adenylyl cyclase stimulation mediated through β_1-adrenoceptors after treatment of human atrial membranes with PTX has also been interpreted as an interruption of the tonic restraint caused by the G_i protein (Grimm *et al*., 1998; Kilts *et al*., 2000). PTX may have merely prevented some G_i protein-evoked tonic inhibition of β_1-adrenoceptor-mediated activation of L-type Ca^{2+} channel conductance (Heubach *et al*., 2001).

The nature of carbachol-evoked tachycardia in PTX-treated mice

Carbachol produced concentration-dependent bradycardia up to 6 μM but 20 and 60 μM tended to enhance sinoatrial rate (Figure 2). PTX treatment not only abolished the bradycardia but uncovered concentration-dependent tachycardia elicited by carbachol (Figure 2). The bradycardia appears to be mediated through M_2 receptors coupled to G_i protein (Caulfield & Birdsall, 1998). To account for the nature of the carbachol-evoked tachycardia, a nicotinic receptor could be involved as seen with vagal stimulation in dogs (Loeb & Vassalle, 1978). The nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) can produce sinoatrial tachycardia in guinea-pig atria, but the effect appears to be produced mainly through a release of noradrenaline from a tyramine-sensitive pool of sympathetic nerve endings (Aramendia & Kaumann, 1967). Our experiments rule out a significant carbachol-induced release of noradrenaline and interaction with β-adrenoceptors because (−)-propranolol did not antagonize the carbachol-evoked tachycardia in atria from PTX-treated mice. Instead, atropine completely reversed the carbachol-evoked tachycardia which is more in line with mediation through a muscarinic receptor. A similar atropine-sensitive and propranolol-insensitive cardioaccelerant effect of carbachol was observed in sinoatrial node cells of PTX-treated chicks by Agnarsson *et al*. (1988), who speculated that an increased Na^+/Ca^{2+} exchange may be involved. It remains an open question which of the 3 G_q-protein-coupled muscarinic receptors (M_1, M_3, M_5–Caulfield & Birdsall, 1998) is involved in the mediation of the carbachol-evoked tachycardia.

The role of murine cardiac α_1-adrenoceptors

Blockade of β_1-adrenoceptors by CGP 20712A alone or in combination with (−)-bupranolol uncovered negative inotropic effects of (−)-adrenaline which were antagonized in surmountable manner by phentolamine suggesting interaction with α-adrenoceptors. Prazosin was more potent than phentolamine, nearly abolishing the negative inotropic responses to (−)-adrenaline (Figures 7 and 8), indicating involvement of an α_1-adrenoceptor. These results are consistent with a considerably higher affinity of prazosin for α_1-adrenoceptors than phentolamine, regardless of α_1-adrenoceptor-subtype, in a variety of systems and species (Michel *et al*., 1995). Because the affinity of prazosin for α_1-adrenoceptors is higher than that of phentolamine, and assuming that both antagonists interact with the same

receptor population, it is not surprising that the joint addition of the two antagonists did not cause detectable additional blockade to that seen with prazosin alone (Figure 7). Our results and conclusions agree with those of Tanaka *et al*. (1995), showing that phenylephrine depresses the contractility of murine ventricle in the presence of propranolol (1 μM) and that this effect is blocked by prazosin (1 μM). As found by us in the presence of CGP 20712A alone or in combination with ICI 118,551, Tanaka *et al*. (1995) also reported negative inotropic effects of noradrenaline in the presence of propranolol on murine ventricle, and suggested the involvement of α_{1A}-adrenoceptors. The same group has recently proposed that the negative inotropic effects of phenylephrine are the result of increased cellular Ca^{2+} extrusion through activation of the Na^+/Ca^{2+} exchanger (Nishimaru *et al*., 2001). Negative inotropic effects of both adrenaline and phenylnephrine, mediated through α_1-adrenoceptors, have also been reported for right ventricular papillary muscles of the rat (Kissling *et al*., 1997). As expected from an α_1-adrenoceptor coupled to G_q protein resistant to PTX, PTX treatment did not modify the negative inotropic potencies of (−)-adrenaline and (−)-noradrenaline when β_1-adrenoceptors are blocked (Figures 5 and 6). The negative inotropic effects, mediated through α_1-adrenoceptors, contrast with the well-known positive inotropic effects mediated through α_1-adrenoceptors in the hearts from a variety of species, including man (Scholz, 1980; Skomedal *et al*., 1985).

Lack of β_3-adrenoceptor-mediated cardiodepression

Gauthier *et al*. (1996; 1998) reported negative inotropic effects of β_3-adrenoceptor-selective agonists on human ventricular preparations under conditions of blockade of β_1- and β_2-adrenoceptors, but this has not been confirmed (Molenaar *et al*., 1997). Harding (1997) and Gong *et al*. (2001) also failed to detect negative inotropic effects with β_3-adrenoceptor-selective agonists on human ventricular myocytes. However, (−)-isoprenaline and a β_3-adrenoceptor-selective agonist cause negative chronotropic effects in spontaneously beating ventricular cardiomyocytes obtained from new-born β_1/β_2-adrenoceptor double knockout mice (Devic *et al*., 2001). In contrast, the negative inotropic effects of (−)-adrenaline and (−)-noradrenaline, which we studied in murine right ventricle, were resistant to blockade by (−)-bupranolol and the β_3-selective antagonist SR59230A, but antagonized by prazosin, inconsistent with mediation through β_3-adrenoceptors but consistent with mediation through α_1-adrenoceptors. The discrepancy between the results of Devic *et al*. (2001) and our results suggests an age-related difference in the function of murine ventricular β_3-adrenoceptors.

Functional antagonism between β_1- and α_1-adrenoceptors

Cardiodepressant effects became apparent at low concentrations of (−)-adrenaline but not of (−)-noradrenaline in the absence of adrenoceptor blocking agents. The negative inotropic effect of (−)-adrenaline is more potent than that of (−)-noradrenaline, whilst the positive inotropic effects of both catecholamines are similar. The positive inotropic effects of (−)-noradrenaline, mediated through β_1-adrenoceptors, appear to conceal some simultaneously occurring negative inotropic effects. Similarily, when the positive inotropic effects of (−)-adrenaline were potentiated by PTX pretreatment, the negative inotropic effects became inconspicuous because they were overlapped by increases in contractility (Figure 10). These observations do not imply that the negative inotropic effects of the catecholamine are mediated through a PTX-sensitive G-protein because in the presence of β blockers PTX treatment did not affect the negative inotropic effects of the catecholamines (Figures 5 and 6).

Blockade of α_1-adrenoceptors with prazosin potentiated the positive inotropic effects of (−)-adrenaline and to a lesser extent also those of (−)-noradrenaline. The catecholamines thus exert physiological antagonistic effects mediated through α_1-adrenoceptors against its ventricular stimulation mediated through β_1-adrenoceptors (Figures 9 and 10).

Possible cardioprotective effects of G_i proteins and α_1-adrenoceptors

The Ca^{2+}-contractures (10 mM) in the presence of high catecholamine concentrations facilitated by PTX treatment could be due to an increased Ca^{2+} load of ventricular myocytes, suggesting that G_i protein tonically protects against Ca^{2+} overload. In addition, α_1-AR stimulation may also reduce Ca^{2+} load of ventricular myocytes, although to a smaller extent than G_i protein, at least in murine ventricle under our conditions. A similar protection against Ca^{2+} overload has been suggested for the negative inotropic effects of adrenaline, mediated through α_1-adrenoceptors in the right ventricular myocardium of the rat (Kissling *et al*., 1997).

Conclusions

Acute cardiostimulation by the physiological catecholamines (−)-adrenaline and (−)-noradrenaline in the sinoatrial node and right ventricle of wild-type mice is exclusively mediated through β_1-adrenoceptors. A negative inotropic effect of (−)-adrenaline, mediated through α_1-adrenoceptors, opposes the positive inotropic effects of (−)-adrenaline mediated through β_1-adrenoceptors under our conditions. In agreement with previous work in left atria from Balb/c mice, no chronotropic and inotropic function for β_2-adrenoceptors was detected in the sinoatrial node and right ventricle of C57BL6 mice, not even after treatment with PTX that eliminated both carbachol-evoked bradycardia and negative inotropic effects, and greatly reduced *in vitro* ADP-ribosylation. PTX treatment tended to increase ventricular positive inotropic effects but not sinoatrial tachycardia elicited by physiological catecholamines through β_1-adrenoceptors, presumably by interrupting some tonic inhibition of G_i protein.

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