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Inverse agonist activities of β-adrenoceptor antagonists in rat myocardium

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1 Negative inotropic effects of several β-adrenoceptor (βAR) antagonists on electrically-stimulated right atria, left atria, right ventricles and left ventricular papillary muscles from reserpine-treated rats were used as a measure of their inverse agonist activities.

2 β_1AR antagonists acebutolol, atenolol and metoprolol, β_2AR antagonist ICI-181,551 and nonselective βAR antagonists alprenolol, nadolol, propranolol and timolol produced negative inotropic effects, which were most marked on the right atria.

3 The nonselective βAR antagonist pindolol did not exhibit inverse agonist activity but inhibited the negative inotropic activities of ICI-118,551, atenolol and propranolol.

4 The negative inotropic effects of lidocaine, nifedipine and pentobarbitone were similar on all the four myocardial preparations.

5 The positive inotropic efficacy of salbutamol on right and left atria but not on right ventricles and papillary muscles was comparable to that of isoprenaline. The antagonist activity of ICI-118,551 against isoprenaline was greater on right atria than on other cardiac regions.

6 β_1AR proteins were expressed in all regions of the heart but of β_2AR were primarily localized in the right atrium.

7 It is concluded that β_2AR play a greater role in right atria than in other cardiac regions and almost all βAR antagonists behave as inverse agonists.

Keywords: ICI-118,551; β_1AR antagonists; nonselective βAR antagonists; βAR proteins; inotropic responses; isoprenaline; salbutamol

Abbreviations: AR, adrenoceptors

Introduction

According to the two-state model of receptor activation (Leff, 1995), a fraction of total G protein-coupled receptors exist in spontaneously active conformational state and can couple to G protein in the absence of ligand (Ehlert, 1986; Lefkowitz *et al*., 1993; Bond *et al*., 1995; Milligan *et al*., 1995). The inhibition of the receptor-mediated functions by antagonists in the absence of the agonist is defined as inverse agonism (Ehlert, 1986). This ligand-independent activity of G protein-linked receptors (Schutz & Freissmuth, 1992) has been demonstrated for benzodiazepine receptors (Hunkeler *et al*., 1981; Braestrup *et al*., 1982; Ehlert, 1986), bradykinin B2 receptors (Leeb-Lundberg *et al*., 1994), δ opioid receptors (Costa & Herz, 1989), muscarinic acetylcholine receptors (Hilf & Jakobs, 1992; Hanf *et al*., 1993), 5-hydroxytryptamine receptors (Barker *et al*., 1994; Westphal & Sanders-Bush, 1994) as well as different adrenoceptors (AR) such as α_2AR (Tian *et al*., 1994), βAR (Gotze & Jakobs, 1994), β_1AR (Mewes *et al*., 1993) and β_2AR (Samama *et al*., 1994; Chidiac *et al*., 1994; 1996; Bond *et al*., 1995).

β_2AR selective antagonist ICI-118,551 (Bilski *et al*., 1983) has been demonstrated to act as an inverse agonist in transgenic mice (Bond *et al*., 1995) and cell lines (Chidiac *et al*., 1994; 1996; Samama *et al*., 1994) overexpressing β_2AR. Several workers have reported differences in the relative densities of β_1AR and β_2AR in atrial and ventricular regions of the heart (Minneman *et al*., 1979; Hedberg *et al*., 1980; Brodde *et al*., 1982; Murphree & Saffitz, 1988; Brodde, 1991; Kitagawa *et al*., 1995) and in the positive inotropic activities of different βAR agonists (Deng *et al*., 1997). It is thus possible that the constitutive activities of β_1AR and β_2AR are not uniform in different regions of the heart. In the present study we determined negative inotropic responses of electrically-stimulated left and sinus node-excised right atria as well as right ventricular and left ventricular papillary muscle preparations from reserpine-treated rats to β_2AR selective antagonist ICI-181,551 (Bilski *et al*., 1983) and a number of β_1AR selective and βAR nonselective antagonists (Hoffman & Lefkowitz, 1996); it was assumed that ligand-independent inverse agonist activity of βAR antagonists should express as negative inotropic response. Data suggest that all βAR antagonists with the exception of pindolol exert inverse agonist activity to varying degree, which is most prominent in the right atria and virtually absent in the left ventricular tissue.

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Methods

Chemicals

Acebutolol, alprenolol, atenolol, ICI-118,551 [erythro-(±)-(α-methyl-indan-4-yloxy)-3-isopropylaminobutan-2-ol], metoprolol, nadolol, pindolol, propranolol, timolol, L-isoprenaline HCl, salbutamol, reserpine, lidocaine, nifedipine, leupeptin and aprotinin were purchased from Sigma, St-Louis, MO,

U.S.A. Anti-β_1AR and anti-β_2AR antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Pentobarbitone and all other high purity chemicals were purchased from BDH, Montreal, QC, Canada.

Animals

Male (350–400 g) Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) were used according to a protocol of the McGill University Animal Care Committee. Animals were maintained at 23°C, 50–70% humidity and a 12 h light/dark schedule (lights on 07.00 h–19.00 h) and fed *ad libitum* rat chow and tap water. In order to deplete endogenous catecholamines, 5 mg kg^{-1} reserpine was injected intraperitoneally 24 h before animals were used for these studies. Rats were decapitated and hearts quickly excised and used for different experiments.

Negative inotropic responses

Left atria, right atria (devoid of sinus node), a strip of the right ventricle (4 × 10 mm) and one left ventricular papillary muscle from reserpine-treated rats were used to determine negative inotropic responses. In some studies, right atria were divided into two identical pieces one of which served as the control and to the other was added 10 μM pindolol 30 min before constructing negative inotropic response-curves to selected βAR antagonists. A small number of studies were also done on tissues from non-reserpinized rats. The tension was recorded by means of Grass force-displacement transducers (FT03C) on a Grass polygraph (Quincy, MA, U.S.A.). The preparations were set up in tissue baths at 37°C in Krebs buffer of the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.18, KH_2PO_4 1.2, $NaHCO_3$ 25, dextrose 11 and EDTA 0.03 (Deng *et al*., 1997). The buffer was gassed with a mixture of 95% O_2 and 5% CO_2. Preparations were stimulated at 1 Hz, 5 ms pulse duration and 1.5 times the threshold voltage (20–30 V) and the tension was adjusted to yield maximal basal contractions. Preparations were allowed to equilibrate for 1 h with changes in Krebs buffer every 15 min.

Positive inotropic responses

Right atria, left atria, right ventricle and left ventricular papillary muscles were set up exactly as described above except that the Ca^{2+} concentration in the buffer was 1.8 instead of 2.5 mM, the temperature of the bath was 32 instead of 37°C and rats were not treated with reserpine. Preparations were allowed to equilibrate for 1 h with changes in buffer before constructing concentration-positive inotropic response curves to isoprenaline and salbutamol. In order to determine the antagonist activity of ICI-118,551 against isoprenaline, left and right atria were divided into two and two strips of right ventricles and two left ventricular papillary muscles were set up; one preparation of each tissue served as the control and to the other was added ICI-118,551 (10, 100 and 1000 nM) 30 min before starting the construction of concentration-positive inotropic response curves to isoprenaline.

Western analysis of β_2AR and β_1AR

Right and left atria and ventricles were homogenized in ice-cold buffer (pH 7.5) containing Tris-HCl (10 mM), EDTA, EGTA and benzamidine (1 M each), 1 μg ml^{-1} each of leupeptin and soybean trypsin inhibitor and phenylmethylsulphonyl fluoride (PMSF) (0.2 mM). The supernatant following homogenization at 3,000 × *g* for 10 min was centrifuged at 100,000 × *g* for 45 min; the membrane pellet was solubilized in the above buffer containing 1% Tween 20 for 1 h on ice. The solubilized proteins were quantified by dye-binding method using bovine serum albumin as the standard. Aliquots (300 μg) of soluble proteins were denatured by boiling for 5 min in Laemmli buffer containing 10% β-mercaptoethanol and resolved on 8% SDS–PAGE gels. The transfer of proteins to membranes, the incubation with rabbit polyclonal anti-βAR antibodies and peroxidase-conjugated goat anti-rabbit IgG antibodies were done as previously described (Peri *et al*., 1995). The immunoreactive bands were visualized by chemiluminescence using a commercial kit (Amersham, Oakville, ON, Canada).

Data analysis and statistics

Negative (denoting inverse agonism) and positive inotropic potencies were calculated as pD_2, which was the negative log of EC_{50} (the molar concentration of the test agent causing 50% of the maximal decrease or increase in effects). Since βAR antagonists generally decreased the contractions of the left atria, right ventricles and left ventricular papillary muscles less than 30%, negative inotropic pD_2 on these preparations was not calculated. The antagonist potency of ICI-118,551 was calculated as pK_B, the negative log of the dissociation constant K_B (molar concentration of the antagonist divided by the dose-ratio minus one) (Besse & Furchgott, 1976). Multiple means were subjected to one-way analysis of variance (ANOVA) followed by Bonferroni test for significance; two means were compared by Student's *t*-test for paired means. A probability of less than 0.05 was assumed to denote a significant difference. Data are presented as means ± s.e.mean.

Results

Basal contractions

The basal control contractions of the four sets of preparations from reserpine-treated rats were significantly different ($P<0.05$) from each other and were as follows: right atria, 644 ± 28 mg ($n=91$); left atria, 552 ± 28 mg ($n=85$); right ventricles, 1004 ± 60 mg ($n=87$); left ventricular papillary muscles, 720 ± 47 mg ($n=90$).

Time-course of negative inotropic responses

Negative inotropic effects were observed 1–2 min after the addition of effective concentrations of βAR antagonists and reached a plateau within 5–10 min. The decrease in the contractile force of all the four preparations following the highest concentration (30 μM) of βAR antagonists used could not be reversed by repeated washes with fresh buffer for at least 60 min.

Negative inotropic effects of lidocaine, nifedipine and pentobarbitone

Lidocaine (Figure 1a), nifedipine (Figure 1b) and pentobarbitone (Figure 1c) caused concentration-dependent decreases in the contraction of all myocardial preparations; there was no significant difference in the negative inotropic effects of these agents on the right atrial, left atrial, right ventricular and left ventricular papillary muscle preparations.

Negative inotropic effects of ICI-118,551

β_2AR antagonist ICI-118,551 caused a concentration-dependent decrease in the basal contractions of right atrial preparations; the negative inotropic effects of ICI-118,551 were similar in preparations from non-reserpinized (Figure 2a) and reserpinized rats (Figure 2b). The negative inotropic pD_2 of ICI-181,551 on right atria from non-reserpinized rats were, respectively, 6.01 ± 0.17 ($n=8$) and 6.08 ± 0.18 ($n=12$). ICI-181, 551 produced minimal effects on the basal contractions of left atria, right ventricles and papillary muscles from both control and reserpine-treated rats (Table 1).

Negative inotropic effects of β_1AR antagonists

Unlike ICI-118,551, the negative inotropic effects of β_1AR antagonist atenolol on right atria and left atria from reserpinized rats (Figure 2d) were less than on preparations from non-reserpinized animals (Figure 2c). Atenolol exerted minimal negative inotropic effects on right ventricles and left ventricular papillary muscles from both non-reserpinized and reserpinized rats (Figure 2c and d).

The negative inotropic potency and efficacy of metoprolol were similar to those of atenolol; however, both atenolol and metoprolol were more efficacious than acebutolol (Table 1).

Negative inotropic effects of nonselective βAR antagonists

All nonselective βAR antagonists studied (alprenolol, nadolol, pindolol, propranolol and timolol) with the exception of pindolol exhibited inverse agonist activities; they caused a greater decrease in the contractions of the right atria than of the other three preparations (left atria, right ventricles and left ventricular papillary muscles) (Figure 3). Alprenolol, nadolol, propranolol and timolol caused significantly greater decrease in the basal contractions of the right atria than did acebutolol (Table 1).

Inhibition of negative inotropic effects of βAR antagonists by pindolol

Pindolol produced minimal effects on the basal contractions of right atria (Figure 4a) and other cardiac regions (Table 1). However, pindolol inhibited the negative inotropic effects of ICI-118,551 (Figure 4b), atenolol (Figure 4c) and propranolol (Figure 4d); this inhibitory effect of pindolol appeared to be less marked against β_1AR antagonist atenolol than against β_2AR antagonist ICI-118,551 and nonselective βAR antagonist propranolol. Effect of pindolol on negative inotropic responses to other βAR antagonists and on other cardiac regions was not studied.

Positive inotropic effects of isoprenaline and salbutamol

Isoprenaline produced concentration-dependent positive inotropic effects on all the four myocardial preparations studied; the rank order of potency and efficacy was: right atria > left aria > right ventricle > left ventricular papillary muscles (Figure 5a). The efficacy of salbutamol on right and left atria was comparable to that of isoprenaline; however, salbutamol exerted minimal positive inotropic effects on right ventricles and left ventricular papillary muscles (Figure 5b).

Antagonism of the positive inotropic effects of isoprenaline by ICI-118,551

ICI-118,551 at a concentration of 100 nM antagonized the positive inotropic effects of isoprenaline on right atria (Figure 6a) but exerted little effect on left atria (Figure 6b), right ventricles (Figure 6c) and papillary muscles (Figure 6d). The pK_B of ICI-118,551 against isoprenaline on right atria was 7.81 ± 0.08 at 100 nM ($n=7$) and did not differ significantly from its pK_B (7.78 ± 0.21) at 10 nM (data not shown). PD_2 values of isoprenaline in the absence and the presence of 100 nM ICI-118,551 were, respectively, 7.21 ± 0.27 and 7.22 ± 0.21 on left atria, 7.13 ± 0.3 and 7.04 ± 0.26 on right ventricles and 6.81 ± 0.03 and 6.78 ± 0.41 on left ventricular papillary muscles; ICI-118,551 did not cause a significant change in pD_2 of isoprenaline on these three preparations. At relatively high concentration (1 μM), ICI-118,551 inhibited the effects of isoprenaline on right and left atria as well as right

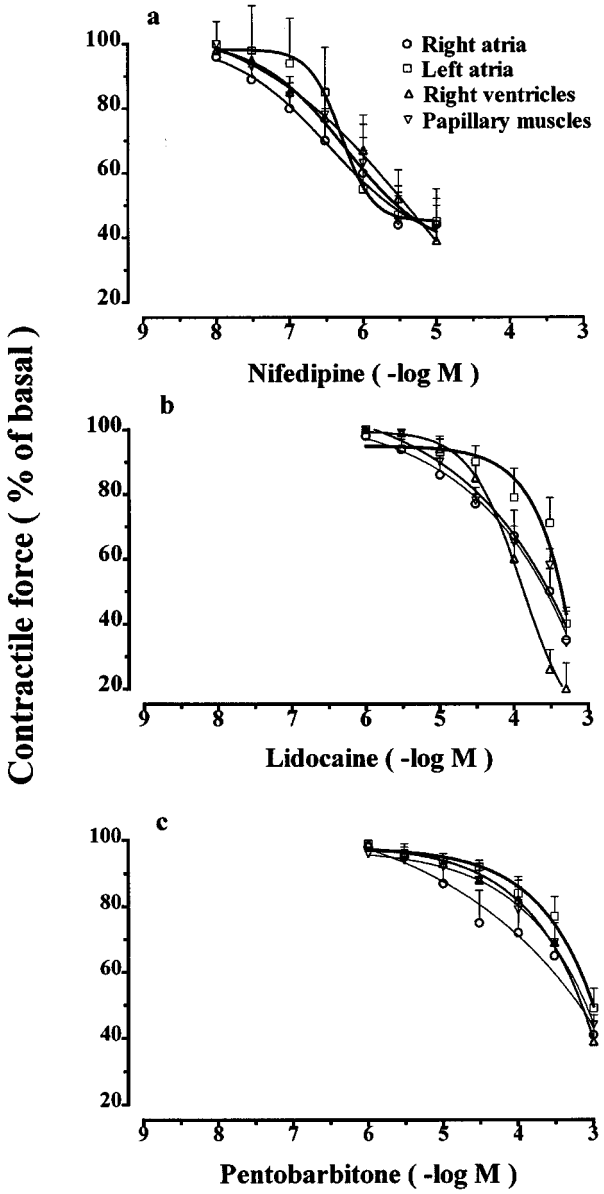

Figure 1 Negative inotropic effects of nifedipine (a), lidocaine (b) and pentobarbitone (c) on electrically-stimulated (1 Hz) right atria, left atria, right ventricles and left ventricular papillary muscles from reserpine-treated rats. Data are means ± s.e.mean of 6–8 separate experiments; all the four preparations in each experiment were from the same animal.

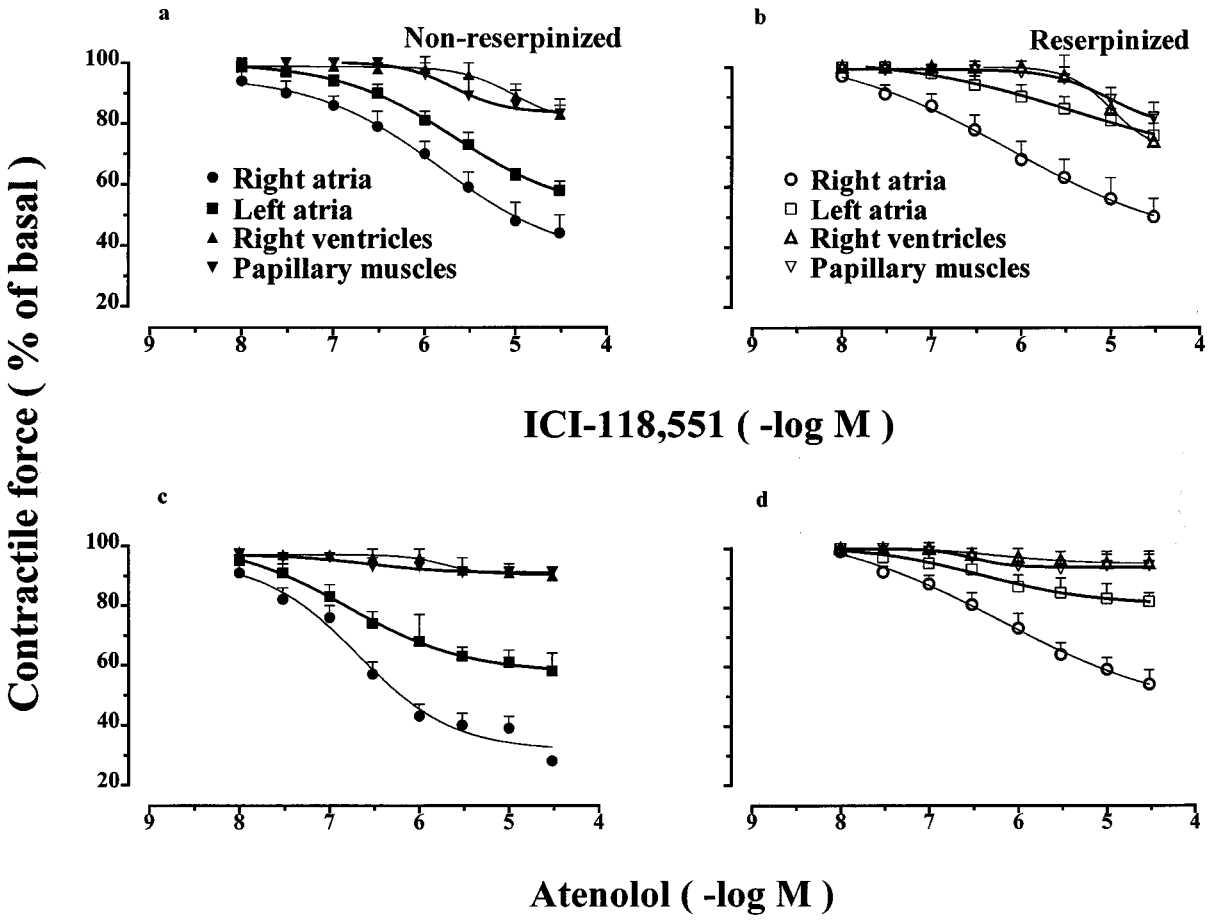

Figure 2 Negative inotropic effects of ICI-118,551 (a and b) and atenolol (c and d) on electrically-stimulated (1 Hz) right atria, left atria, right ventricles and left ventricular papillary muscles from non-reserpinized (a and c) and reserpinized (b and d) rats. Data are means ± s.e.mean of 8–12 separate experiments; all the four preparations in each experiment were from the same animal.

Table 1 Negative inotropic effects of b-adrenoceptor antagonists on right atria (RA), left atria (LA), right ventricles (RV) and papillary muscles (PM) from reserpine-treated rats

		RA	*RA*	*LA*	*RV*	*PM*
Antagonists	*n*	pD_2	*Contractile force (% of basal) at 30 μM antagonists*			
Acebutolol	6	NC	73±9	87±4	90±5	95±6
Atenolol	9	6.17±0.18	54±5	82±4	95±4	94±4
Metoprolol	8	6.03±0.20	57±6	84±8	95±2	87±6
ICI-118,551	12	6.19±0.21	50±6	77±4	76±8	83±5
Alprenolol	6	5.88±0.19	58±5	77±3	68±11	65±5
Nadolol	7	6.40±0.30	56±9	80±5	91±8	93±4
Pindolol	7	NC	88±6	83±4	86±4	82±5
Propranolol	7	6.46±0.31	48±9	74±5	59±7	90±5
Timolol	6	6.48±0.25	55±7	83±8	76±7	88±7

Preparations were set up at 37°C and stimulated at 1 Hz. Right atria were devoid of sinus node. Since the maximal decrease in basal contractions of left atria, right atria and left ventricular papillary muscles at the highest concentration (30 mM) of the antagonists used was generally less than 30%. pD_2 was not calculated; NC, not calculated. *Significantly ($P<0.05$) different from all other values in the same column but not from each other; †Significantly ($P<0.05$) different from all other values in the same row, ‡Significantly ($P<0.05$) different from all other values in the column except that for alprenolol.

ventricles but the shift in concentration-response curves to isoprenaline was not parallel (data not shown).

Western analysis of βAR

Distinct band corresponding to β_2AR at 47 kDa region was detected in right atrium and to a lesser extent in left atrium; this 47 kDa band could not be detected in right or left ventricular tissue (Figure 7a). In contrast β_1AR proteins were detected in both right and left atria and ventricles in greater abundance than β_2AR proteins (Figure 7b).

Discussion

Inverse agonist activity of ICI-118,551 in the heart of transgenic mice (Bond *et al*., 1995) and of several other βAR antagonists in cells overexpressing β_2AR (Chidiac *et al*., 1994;

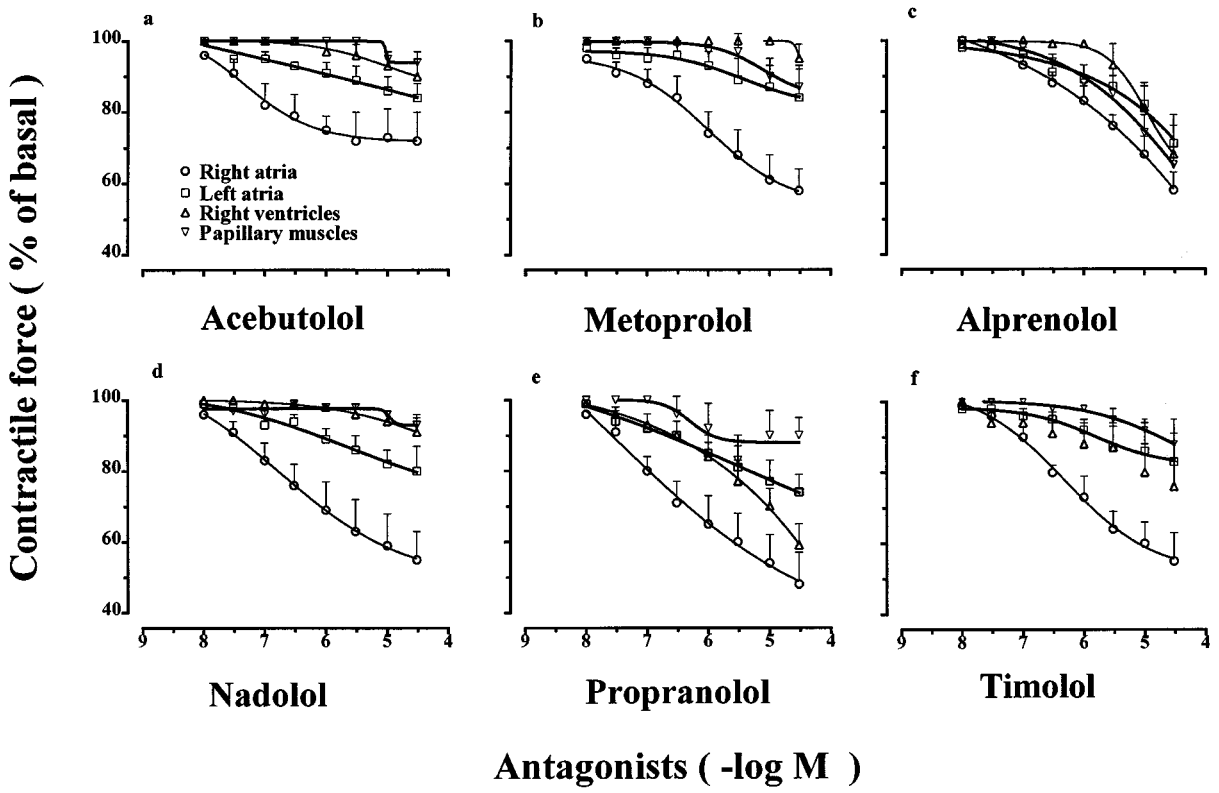

Figure 3 Negative inotropic effects of different β-adrenoceptor antagonists on electrically-stimulated (1 Hz) right atria, left atria, right ventricles and left ventricular papillary muscles from reserpine-treated rats. Data are means$\pm$s.e.mean of 6–12 separate experiments; all the four preparations in each experiment were from the same animal.

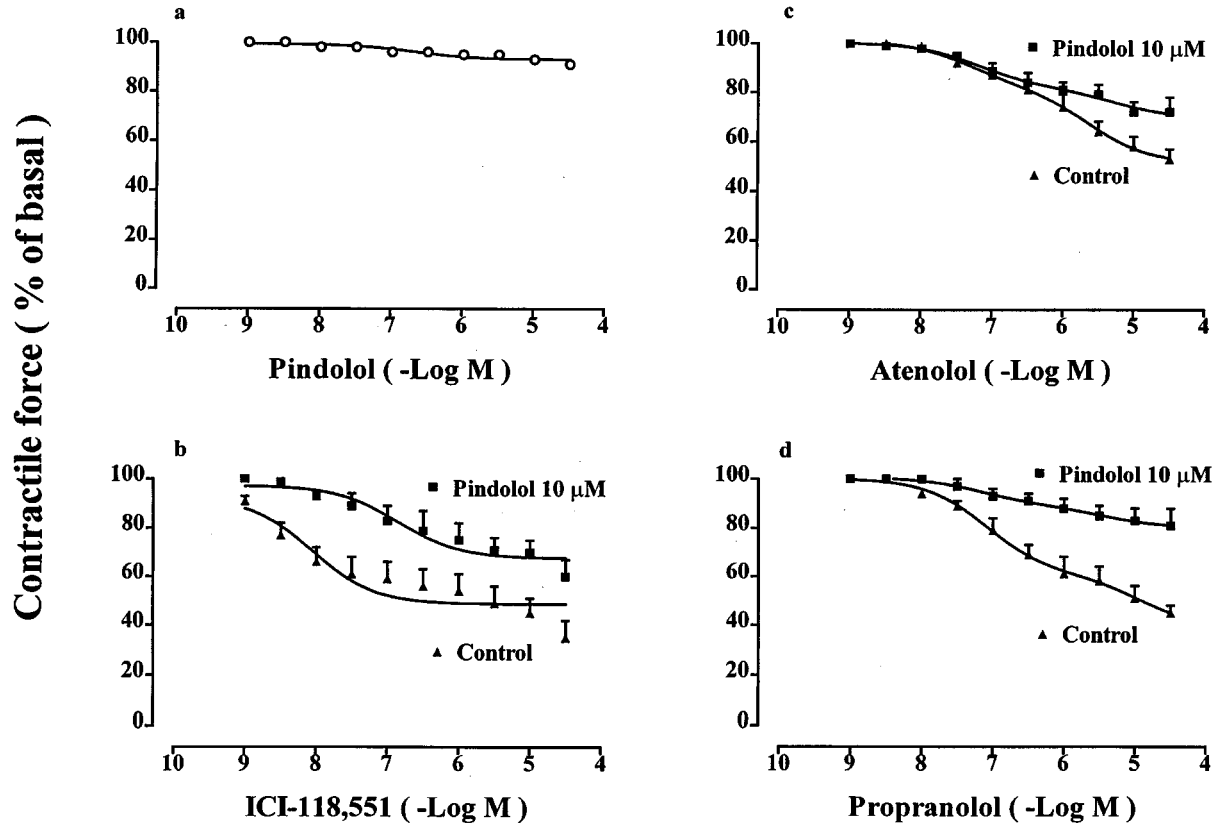

Figure 4 Inhibition of the negative inotropic effects of ICI-118,551, atenolol and propranolol by pindolol on electrically-stimulated right atria from reserpine-treated rats: (a) concentration-response curve to pindolol; in b, c and d right atria were divided into two, one of which served as the control and to the other was added 10 μM pindolol 30 min before constructing the concentration-response curve to ICI-118,551 (b), atenolol (c) and propranolol (d). Data are means$\pm$s.e.mean of 7–9 experiments.

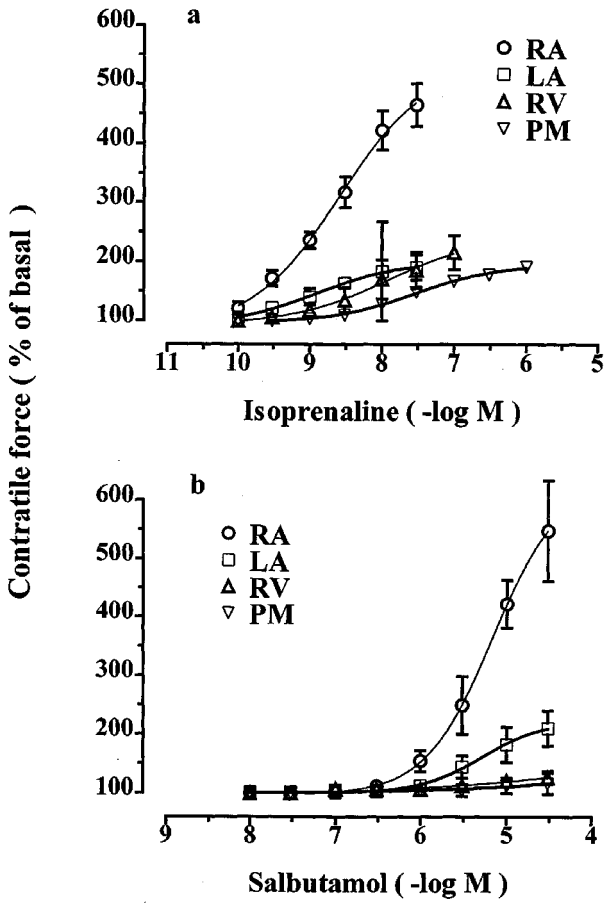

Figure 5 Positive inotropic effects of isoprenaline (a) and salbutamol (b) on electrically-stimulated (1 Hz) right atria, left atria, right ventricles and left ventricular papillary muscles from non-reserpinized rats. Data are means ± s.e.mean of 7–8 separate experiments; all the four preparations in each experiment for each of the two agonists were from the same animal.

1996) has been previously demonstrated. The primary objective of this study was to determine if βAR antagonists exert inverse agonist activity in native rat myocardium at physiological concentrations of β_1AR and β_2AR. Because an activation of βAR increases myocardial contractions, we assumed that the inverse activity of βAR antagonists would express as negative inotropic response.

The present study revealed that different βAR antagonists with the exception of pindolol exhibited inverse agonist activities; however they all produced greater negative inotropic effects on the right atria than on other myocardial preparations (left atria, right ventricles and left ventricular papillary muscles). Since nifedipine, lidocaine and pentobarbitone caused comparable decreases in the contractions of the atrial and ventricular preparations (Figure 1), it would appear that the differences in the effects of βAR antagonists on right atria and other preparations reflect sensitivities of βAR to inverse agonist activities of different agents and not differences in their 'quinidine-like' or 'membrane-stabilizing' activities (Hoffman & Lefkowitz, 1996). This inference is also supported by the data that the nonselective βAR antagonist pindolol caused little decrease in the basal contractions of all the four myocardial preparations studied.

The negative inotropic effect of ICI-118,551 was not modified by reserpine treatment (Figure 2a and b) in conformity with other reports (Bond *et al*., 1995). It would thus appear that β_2AR in their native state are not under the influence of endogenous catecholamines, as suggested by others (Bryan *et al*., 1981). Moreover, endogenous catecholamines do not seem to equally modulate basal functions of β_1AR in different regions of the heart since the negative inotropic effect of atenolol on right and left atria but not on the ventricular preparations was decreased by reserpine treatment (Figure 2c and d). Because myocardial preparations from reserpine-treated rats were used as a measure of inverse agonism, it could be assumed that the negative inotropic responses of βAR antagonists were minimally caused by antagonizing the effects of endogenous catecholamines. This inference is also supported by the data that nonselective βAR antagonist pindolol exerted little negative inotropic effect (Figure 4a, Table 1).

We studied β_2AR selective antagonist ICI-118,551 (Bilski *et al*., 1983), β_1AR selective antagonists metoprolol, atenolol and acebutolol, and nonselective βAR antagonists alprenolol, nadolol, pindolol, propranolol and timolol (Hoffman & Lefkowitz, 1996). Since both β_1AR and β_2AR antagonists exerted negative inotropic effects, it would appear that both β_1AR and β_2AR are susceptible to inverse agonist activity. The findings that the inverse agonist activities of βAR antagonists were greater on the right atria than on other preparations (left atria, right ventricles, papillary muscles) suggest that the constitutive activity of βAR is tissue-specific. The greater susceptibility of right atria than of other cardiac regions to inverse agonist activities of βAR antagonists seems to be related to relative distribution of β_1AR and β_2AR as well as to a possible greater constitutive activity of β_2AR than of β_1AR. It has been reported that ICI-118,551 did not decrease whole-cell L-type calcium currents in guinea-pig ventricular cardiomyocytes although both atenolol and propranolol exhibited inverse agonist activities (Mewes *et al*., 1993); it is very possible that a lack of inverse agonist activity of ICI-118, 551 in these ventricular cardiomyocyte preparations reflects sparse expression of β_2AR.

Studies using different regions of the myocardium from humans (Brodde, 1991), rabbits (Brodde *et al*., 1982), cats (Hedberg *et al*., 1980), guinea-pigs (Hedberg *et al*., 1980), rats (Hancock *et al*., 1979; Minneman *et al*., 1979; Kitagawa *et al*., 1995) and dogs (Murphree & Saffitz, 1988) have consistently demonstrated a much higher concentration of β_1AR than of β_2AR. β_2AR constitute approximately 20% of the total in right atria and are either undetectable (Hedberg *et al*., 1980) or expressed at much lower concentration in other regions (Brodde *et al*., 1982; Kitagawa *et al*., 1995). In conformity with these data, we found that β_2AR protein was expressed primarily in the right atria whereas β_1AR proteins were expressed in greater abundance in all myocardial regions including the right atria (Figure 7). A much greater positive inotropic efficacy of salbutamol on right atria than on left atria and minimal effects on right ventricles and papillary muscles (Figure 5) as well as a greater antagonist activity of ICI-118,551 against isoprenaline on right atria than on left atria and left ventricular papillary muscles (Figure 6) also suggest a greater functional role of β_2AR on right atria than in other cardiac regions of the rat. Since the inverse agonist efficacy of β_2AR antagonist ICI-118,551 on right atria was comparable to that of β_1AR antagonists metoprolol and atenolol as well as βAR nonselective antagonist propranolol despite a relatively lower concentration of β_2AR, it would appear that the relative constitutive activity of β_2AR exceeds that of β_1AR.

The reported inverse agonist potency of ICI-118,551 on myocardial β_2AR in transgenic mice (Bond *et al*., 1995) and of nonselective βAR antagonists in cells overexpressing β_2AR (Chidiac *et al*., 1994; 1996) is much higher than found in this

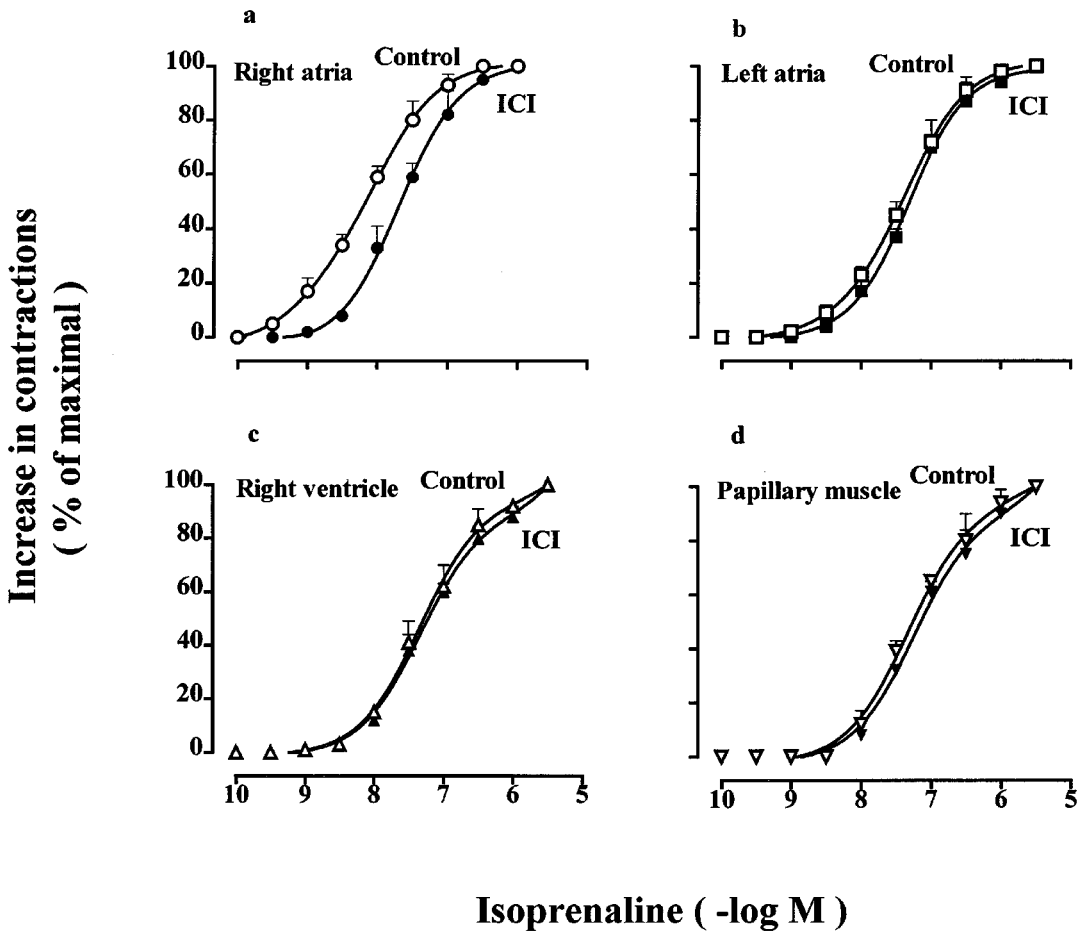

Figure 6 Effect of ICI-118,551 (ICI, 100 nM) on electrically stimulated (1 Hz) right atria (a), left atria (b), right ventricles (c) and left ventricular papillary muscles (d) from non-reserpinized rats. Two identical pieces of each tissue from the same rat were used; one served as the control and to the other was added 100 nM ICI-118,551, 30 min before starting the construction of concentration-response curves to isoprenaline. Data are means ± s.e.mean of 6–8 separate experiments.

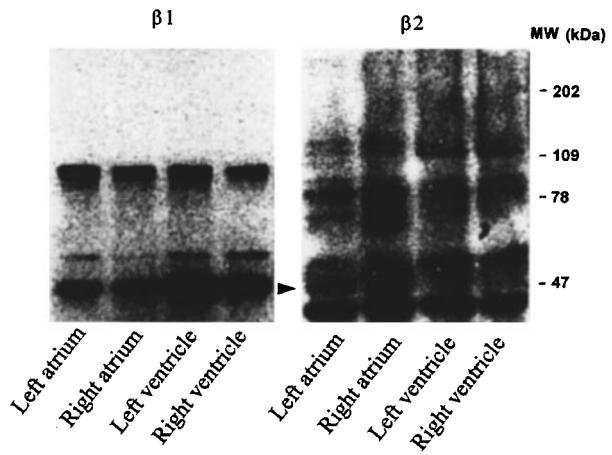

Figure 7 Western blots of β_1- and β_2-adrenoceptors in left atria, right atria, left ventricles and right ventricles of rats. Aliquots (300 μg) of detergent-soluble proteins were resolved by SDS–PAGE followed by immunoblotting using receptor isoform-specific polyclonal antibodies. The arrows point to the immunoreactive bands of β_1- and β_2-adrenoceptor proteins. For sufficient protein yield from atria, four non-reserpinized rats were killed and the four cardiac regions pooled for one experiment.

study. A possible explanation of this disparity in the inverse agonist potencies might be related to much higher concentration of receptors following over expression than in native tissues. Furthermore, Bond *et al.* (1995) found alprenolol to behave as a neutral antagonist in mouse myocardium overexpressing β_2AR although other workers (Chidiac *et al.*, 1994) found that all βAR antagonists tested (alprenolol, propranolol, timolol and pindolol, labetalol, dichloroisoprenaline) exhibited inverse agonist activities. The data of this study are in conformity with that reported for cells overexpressing β_2AR (Chidiac *et al.*, 1994). Since the negative inotropic effect of alprenolol was comparable to that of ICI-118,551, propranolol and metoprolol, it would seem that alprenolol can act as an inverse agonist at concentrations much higher than those utilized by Bond *et al.* (1995) in their studies.

In the present study using rats, on the other hand, pindolol was found to behave as a 'neutral' antagonist. Although pindolol exerted little negative inotropic effect, it significantly reduced the inverse agonist activities of β_2AR antagonist ICI-118,551, β_1AR antagonist atenolol and nonselective βAR antagonist propranolol (Figure 4); these data also suggest that the negative inotropic activity of ICI-118,551, atenolol and propranolol and possibly of other βAR antagonists reflects inverse agonist activities of these agents rather than an antagonism of endogenous ligands.

In summary, data of this study provide evidence for an inverse agonist activity of βAR antagonists in native myocardial tissues. Since the negative inotropic activities of different βAR antagonists considerably vary in different cardiac regions but are present even after the depletion of endogenous catecholamines, it would appear that myocardial

depression following clinical use of these agents might in part reflect their inverse agonist activities; the magnitude of this depressant effect might vary in different cardiac regions depending upon βAR concentrations. The data of this study do not elaborate on the 'two-state' model of receptor activation (Leff, 1995) but are consistent with this hypothesis and provide additional evidence for constitutive activity of βAR receptors in native cardiac tissues.

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