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CHANGES IN THE *IN VITRO* PHARMACODYNAMIC PROPERTIES OF METOPROLOL IN ATRIA ISOLATED FROM SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY

1. The present study addressed possible changes in the dissociation constant of metoprolol and its inverse agonist activity in spontaneously hypertensive rats (SHR). In addition, a possible correlation between cardiac hypertrophy and the inverse agonist activity of metoprolol was explored.

2. In order to determine the dissociation constant (expressed as the pK_b) of metoprolol, a cumulative concentration–response curve to noradrenaline was constructed in the absence or presence of metoprolol (0.1, 1 or 10 μmol/L). In a second experiment, a cumulative concentration–response curve to metoprolol was constructed to determine its inverse agonist activity.

3. The ventricular weight of SHR was significantly greater compared with Wistar-Kyoto (WKY) rats. A rightward shift of the concentration–response curve to noradrenaline was observed in SHR compared with WKY rats. The pK_b of metoprolol was smaller in SHR compared with WKY rats (6.35 ± 0.14 *vs* 6.99 ± 0.12, respectively; $P < 0.05$). No difference was observed in the maximal response (E_{max}) of the concentration–time effect of metoprolol in WKY rats and SHR (-29.1 ± 7.1 *vs* $-28.2 \pm 8.5\%$, respectively; $n = 6$ for both). However, the concentration of metoprolol eliciting a half-maximal effect (expressed as the pEC_{50}) was significantly smaller in SHR compared with WKY rats (4.82 ± 0.07 *vs* 5.29 ± 0.13, respectively; $n = 6$; $P < 0.05$). Although a significant correlation ($r = -0.876$) between the ventricular weight/bodyweight (VW/BW) ratio and the pEC_{50} of the chronotropic effect of metoprolol was found, no relationship ($r = -0.257$) was found between the VW/BW ratio and E_{max}.

4. In summary, the present study provides the first evidence of a change in the *in vitro* pharmacodynamic properties of metoprolol in SHR. The sympathetic overactivity present in SHR not only reduces the positive chronotropic effect of noradrenaline, but also diminishes the constant dissociation of metoprolol from atrial β_1-adrenoceptors and its inverse agonist activity. A significant correlation between the VW/BW ratio and the inverse agonist potency of metoprolol was found, suggesting a possible link between cardiac hypertrophy and the reduction of the inverse agonist activity of metoprolol.

Key words: cardiac hypertrophy, chronotropic effect, dissociation constant, inverse agonist activity, metoprolol, spontaneously hypertension.

INTRODUCTION

The responsiveness of cardiac β-adrenoceptors is diminished in different models of experimental hypertension, including spontaneously hypertensive rats (SHR),[1] aortic coarctated,[2] DOCA-salt and renal hypertensive rats.[3] In addition, changes at the post-receptor level, such as adenyl cyclase desensitization, have also been observed.[4] Among the different mechanisms involved in these alterations, it has been proposed that sympathetic nervous system overactivity present in these experimental models may play a mayor role.

Despite the existence of conflicting results with regard to β-adrenoceptor density,[5] there seems to be agreement with respect to β-adrenoceptor affinity, which is similar between SHR and Wistar-Kyoto (WKY) rats.[5]

Metoprolol is a cardioselective β-adrenoceptor antagonist used in the treatment of several cardiovascular pathologies, including arterial hypertension and heart failure. This drug, like others β-adrenoceptor antagonists, exerts an inverse agonist action at different sites of the heart.[6] This pharmacodynamic property makes the beta-blocker with inverse agonist activity an attractive tool for preventing a system from hazards that will be developed by oversignalling of β-adrenoceptors.[7] Furthermore, inverse agonists have a more pronounced effect on the upregulation of desensitized β-adrenoceptor-mediated signalling than do neutral antagonists.[7] It is suggested that β-adrenoceptor desensitization observed in heart failure can alter the inverse agonist activity of β-adrenoceptor antagonists.[8]

However, although β-adrenoceptor activity in response to adrenoceptor agonists has been studied extensively in SHR,[9–12] to the best our knowledge the pharmacodynamic properties of beta-blockers have been rarely investigated in experimental models of hypertension. In a previous study, we found that the dissociation constant of metoprolol and its inverse agonist activity were not affected in aortic coarctated rats at an acute and chronic hypertensive stage.[2,13]

Thus, the aims of the present study were: (i) to study the dissociation constant and the inverse agonist activity of metoprolol in atria isolated from WKY rats and SHR; and (ii) to explore the

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possible correlation between cardiac hypertrophy and the inverse agonist activity of metoprolol.

METHODS

Experimental design

Twelve-week-old male WKY rats and SHR were used. Animal experiments were performed in accordance with the *Principles of Laboratory Animal Care* published by the National Institutes of Health.[14]

Animals were killed by cervical dislocation and hearts were removed quickly and placed in a Petri dish filled with physiological salt solution composed of (in mmol/L): NaCl 120; KCl 4.8; KH_2PO4 1.2; $CaCl_2.2H_2O$ 1.6; $MgSO_4$ 1.33; dextrose 10. The combined atria were then carefully dissected free of connective tissues and ventricles, mounted in an organ bath and connected to a Grass force transducer (Grass Instrument Co., Quincy, MA, USA). The temperature of the bath was maintained at 37°C and the bathing solution was bubbled with 95% O_2 and 5% CO_2. A tension of 1 g was applied to the atria.

The preparation was allowed to equilibrate for at least 45 min, during which time it was washed every 15 min. Drugs were added when the spontaneous rate of beating changed no more than 5% within a 10 min period.

To determine the dissociation constant of metoprolol, a cumulative concentration–response curve to noradrenaline was constructed in the absence or presence of metoprolol (0.1, 1 or 10 μmol/L). Cumulative concentration–response curves to noradrenaline were constructed after 30 min of incubation with metoprolol. A single cumulative concentration–response curve to noradrenaline was determined in each pair of atria.

To determine the inverse agonist activity of metoprolol, a cumulative concentration–response curve to metoprolol was constructed using concentrations of 10^{-8}, 10^{-7}, 3×10^{-6}, 10^{-6}, 3×10^{-5}, 10^{-5}, 3×10^{-4} and 10^{-4} mol/L.

Additional determinations

The weight of the left and right ventricles combined was determined with a precision balance (Ohaus Adventurer, Pine Brook, NJ, USA). The protein content of the left ventricle was quantified according to the method of Lowry *et al.*[15]

Data analysis

In the study of the dissociation constant of metoprolol in isolated atria, the negative decadian logarithm of the effective concentration yielding a half-maximal response (pEC_{50}) and the maximal effect (E_{max}) from the concentration–response curves to noradrenaline (control curve and curves construction in the presence of 0.1, 1 and 10 μmol/L of metoprolol) were calculated from non-linear regression fits of the data to a sigmoidal concentration–response curve.

Then, the pEC_{50} data obtained from the concentration–response curves to noradrenaline versus the metoprolol concentration plot were adjusted by non-linear regression according to the equation designed by Lew and Angus.[16]

The inverse agonist activity of metoprolol was determined by estimation of the pEC_{50} and E_{max} of the concentration–response curve to metoprolol using a non-linear regression method fitting the data to a sigmoidal concentration–response curve.

Statistics

Regression analysis and statistical tests were performed using standard software (GraphPad Prism v. 3.02 for Windows; GraphPad Software, San Diego, CA, USA). Normal distribution of the data and variables were verified using the Kolmogorov–Smirnov test. Statistical analysis was performed with Student's *t*-test. The correlation between the ventricular weight to bodyweight (VW/BW) ratio and parameters of the inverse agonist activity of metoprolol (pEC_{50} and E_{max}) was studied by means of Pearson's test.

Data are expressed as the mean±SEM. Statistical significance was defined as $P < 0.05$.

Drugs

The following drugs were used: metoprolol (a generous gift from Novartis Laboratory, Rueil-Malmaison, France) and noradrenaline (Sigma, St Louis, MO, USA).

RESULTS

No differences were observed in the total weight of SHR and WKY rats (Table 1). Ventricular weight was significantly greater in SHR compared with WKY rats (Table 1). In addition, a greater protein content was found in ventricles of SHR compared with the control (WKY rats) group (Table 1).

Basal values of spontaneous atrial beating were 219 ± 15 and 229 ± 14 b.p.m. in WKY rats and SHR, respectively ($n = 20$ for both). The chronotropic response of isolated atria to noradrenaline stimulation was altered in SHR compared with WKY rats. A rightward shifting of the concentration–response curve to noradrenaline was observed in SHR (Fig. 1). The pEC_{50} and E_{max} of the chronotropic effect of noradrenaline were significantly smaller in SHR compared with WKY rats (Table 2).

Table 1 Ventricular weight to total bodyweight ratio and ventricular protein content in Wistar-Kyoto and spontaneously hypertensive rats

	WKY rats ($n = 20$)	SHR ($n = 20$)
Total bodyweight (g)	166 ± 7	179 ± 8
VW (mg)	475 ± 26	768 ± 24*
VW/BW ratio (mg/g)	2.89 ± 0.13	4.37 ± 0.14*
Ventricular protein content (μg/mg)	187 ± 4	244 ± 4*

Data are the mean±SEM. *$P < 0.05$ compared with Wistar-Kyoto (WKY) rats.

SHR, spontaneously hypertensive rats; VW, ventricular weight; BW, bodyweight.

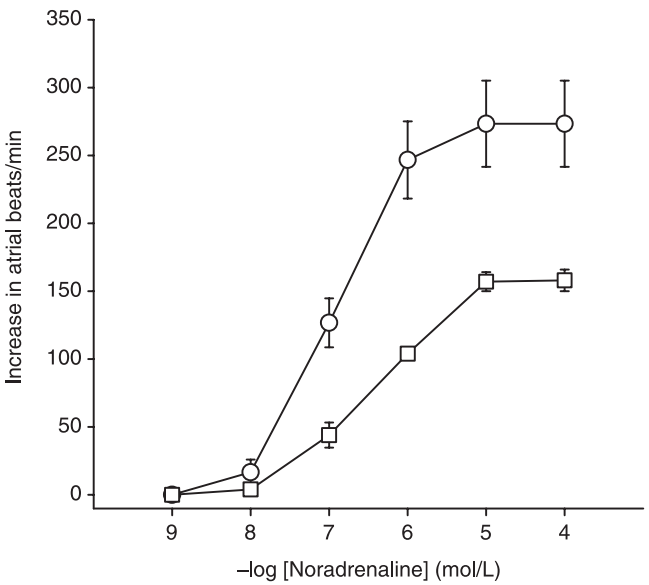

Fig. 1 Concentration–response curve for the chronotropic effect of noradrenaline in atria isolated from Wistar-Kyoto (○) and spontaneously hypertensive (□) rats. Data are the mean±SEM of five rats.

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Table 2 pEC_{50} and E_{max} (maximal response) of concentration–response curves to the chronotropic effect of noradrenaline in atria isolated from Wistar-Kyoto and spontaneously hypertensive rats

	Concentration–response curve of noradrenaline			
	WKY rats		SHR	
	pEC_{50}	E_{max} (b.p.m.)	pEC_{50}	E_{max} (b.p.m.)
Control	6.93 ± 0.02	275 ± 31	6.27 ± 0.13*	166 ± 9*
+ Metoprolol				
0.1 μmol/L	6.81 ± 0.08	241 ± 38	6.07 ± 0.09*	163 ± 13
1 μmol/L	5.74 ± 0.02	214 ± 14	5.59 ± 0.14	147 ± 9*
10 μmol/L	5.11 ± 0.03	229 ± 17	4.81 ± 0.05*	144 ± 18*

Data are the mean±SEM (n = 5). *P < 0.05 compared with Wistar-Kyoto (WKY) rats.

SHR, spontaneously hypertensive rats.

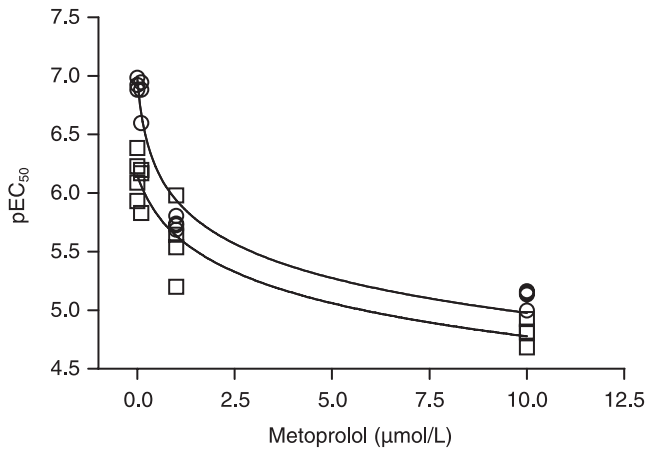

Fig. 2 Effect of metoprolol on the pEC_{50} value for noradrenaline in Wistar-Kyoto (○) and spontaneously hypertensive (□) rats. Metoprolol potency data were analysed using non-linear regression to yield pK_b estimates.

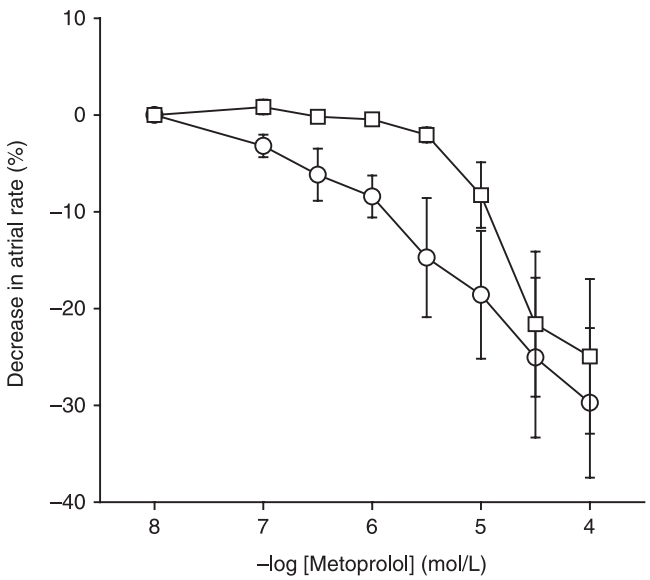

Fig. 3 Concentration–response curve for the chronotropic effects of metoprolol in atria isolated from Wistar-Kyoto (○) and spontaneously hypertensive (□) rats. Data are the mean±SEM of six rats.

Figure 2 shows the plot of the pEC_{50} of the chronotropic effect of noradrenaline (listed in Table 2) as a function of metoprolol concentration in SHR and WKY rats. The data fitted well to the equation in both experimental groups (r^2 = 0.952 and 0.889 for WKY rats and SHR, respectively). The pK_b was determined by means of a non-linear regression and a smaller pK_b was observed in SHR compared with WKY rats (6.35 ± 0.14 *vs* 6.99 ± 0.12, respectively; P < 0.05).

Figure 3 shows the concentration–response curve to metoprolol obtained in atria isolated from WKY rats and SHR. No differences were found in the E_{max} of the chronotropic effect of metoprolol in WKY rats and SHR (–29.1 ± 7.1 *vs* –28.2 ± 8.5%, respectively; n = 6 for both). However, the pEC_{50} of metoprolol was significantly smaller in SHR compared with that in WKY rats (4.82 ± 0.07 *vs* 5.29 ± 0.13, respectively; n = 6 for both; P < 0.05).

Finally, the relationship between the VW/BW ratio and the inverse agonist activity of metoprolol was examined. Although a significant correlation (r = –0876) between the VW/BW ratio and the pEC_{50} of the chronotropic effect of metoprolol was found (Fig. 4a), no relationship (r = –0257) existed between the VW/BW ratio and E_{max} (Fig. 4b).

DISCUSSION

Many studies on changes in cardiac β-adrenoceptors have been performed in SHR, showing decreased, unchanged or increased

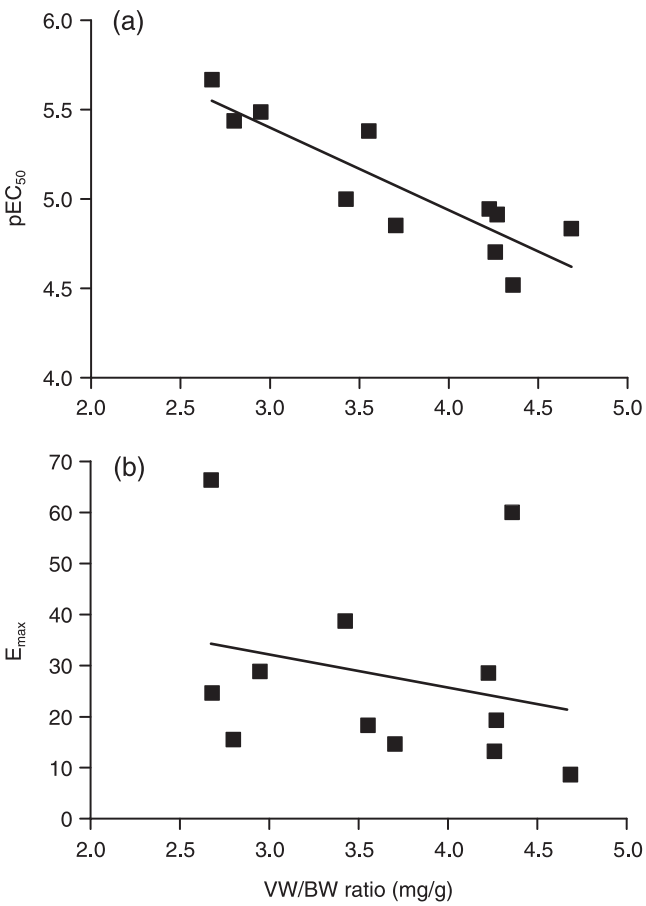

Fig. 4 Correlation between the ventricular weight to bodyweight (VW/BW) ratio and the inverse agonist parameters of metoprolol, namely pEC50 (a) and E_{max} (b), in Wistar-Kyoto and spontaneously hypertensive rats (pooled data).

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receptor density.[5] In addition, although most of the studies did not find differences in ligand affinity to cardiac β-adrenoceptors between SHR and WKY rats,[5] one report showed a decreased affinity in 30-week-old SHR.[17] Conversely, post-receptor events, such as desensitization of adenyl cyclase, are also implicated in the impairment of β-adrenoceptor responsiveness observed in SHR.[4] However, it is important to mention that previous studies were performed only in ventricular myocytes of SHR[5] and, to the best our knowledge, the existence of changes in the density and ligand properties of atrial β_1-adrenoceptors remains unknown in this experimental model of hypertension.

In summary, although conflicting results exist with regard to the mechanisms involved in the blunted response of cardiac β-adrenoceptors in SHR, all the studies conducted have found an impairment of the inotropic and chronotropic effect to β-adrenoceptor stimulation in both ventricular and atrial preparations.[9–12,18] These results are compatible with the reported increase in cardiac sympathetic outflow in genetic hypertension.[19] In the present study, we also found a diminished chronotropic response to noradrenaline in atria isolated from SHR.

Surprisingly, changes in the *in vitro* pharmacodynamic properties of β-adrenoceptor antagonists have rarely been studied in experimental hypertension. Doggrell and Surman[20] have demonstrated that the dissociation constant of bisoprolol to left atrial β_1-adrenoceptors was not altered in SHR.

Under the present experimental conditions, we found a diminished dissociation constant of metoprolol to atrial β_1-adrenoceptors in SHR compared with WKY rats, suggesting that the affinity of metoprolol to the receptor is reduced in genetically hypertensive rats. It is well known that receptors exist in an active and inactive conformation in native tissues and agonists have different degrees of affinity for both receptor stages.[21] Therefore, the altered dissociation constant of metoprolol observed in SHR could be a consequence of changes in the proportion of atrial active/inactive β_1-adrenoceptors.

The *in vitro* chronotropic effect of metoprolol was also studied in isolated atria. Metoprolol, like other β-adrenoceptor antagonists, has a non-uniform inverse agonist action in human and rat myocardium,[6,7] showing greater efficacy in the right atria than in other cardiac tissues. Moreover, metoprolol has a greater inverse agonist efficacy with respect to other β-adrenoceptor antagonists.[7,22]

When studying the inverse agonism of metoprolol, it is important to ensure that the negative chronotropic effect is not due to contamination by endogenous catecholamines or to a membrane-stabilizing action. Several studies have demonstrated that the effect of endogenous catecholamine contamination is negligible with regard to the *in vitro* negative chronotropic and inotropic responses to β-adrenoceptor antagonists.[6,7,22,23] The negative inotropic effect of metoprolol is also not due to its weak membrane-stabilizing activity.[6]

In the present study, although metoprolol induced an *in vitro* negative chronotropic effect in both SHR and WKY rats, its inverse agonist activity was reduced in SHR compared with WKY rats. In both experimental groups, the negative chronotropic potency of metoprolol was much lower than its dissociation constant from β_1-adrenoceptors. The present findings are in agreement with previous studies by other authors.[6,23] A lower than expected inverse agonist potency of β_1- and β_2-adrenoceptor antagonists was observed in these reports.[6,23] Although the exact reason for the low inverse agonist potency is not clear, it is suggested that this finding could be explained by the low constitutive activity of β_1-adrenoceptors in native tissues.[23]

Conversely, previous studies by other authors have shown that the constitutive activity of β_1-adrenoceptor is weaker than that of β_2-adrenoceptors.[23,24] Metoprolol is only relatively specific for β_1-adrenoceptors, because it has a 30-fold selectivity for β_1-adrenoceptors over the β_2-adrenoceptor subtype.[25] Taking this into consideration, another explanation for the lower than expected inverse agonist potency of metoprolol could be that blockade of β_2-adrenoceptors by metoprolol may contribute to its inverse agonist activity.

In the present study, the inverse agonist potency (expressed as the pEC_{50}) for the *in vitro* chronotropic effect of metoprolol was significantly lower in atria isolated from SHR compared with WKY rats. Maack *et al*.[8,22] have demonstrated that desensitization of cardiac β-adrenoceptors in heart failure could alter the inverse agonist activity of β-adrenoceptor antagonists and also established that β_1-adrenoceptor downregulation may reduce the inverse agonist response. In a previous study, we found that the inverse agonist properties of metoprolol were modified by ageing, but not by the hypertensive stage induced by aortic coarctation.[13] It is well known that ageing is associated with a sustained increase of serum noradrenaline levels and a blunted β-adrenoceptor response to catecholamines owing to β_1-adrenoceptor downregulation.[26]

Therefore, the present results suggest that desensitization of β-adrenoceptor in SHR not only affects the activity of total agonists, such as noradrenaline, but also reduces the chronotropic effect of inverse agonists.

Finally, a possible correlation between the degree of inverse agonist activity of metoprolol and the VW/BW ratio was examined. The VW/BW ratio has been defined as a parameter of cardiac hypertrophy.[9] A greater VW/BW ratio and ventricular protein content were observed in SHR, suggesting the presence of cardiac hypertrophy in the hypertensive group. We found a significant correlation between the pEC_{50} of the chronotropic effect of metoprolol and the VW/BW ratio. Animals with a greater VW/BW ratio showed a lower inverse agonist potency of metoprolol. This finding could be attributed to the overactivity of the sympathetic nervous system observed in SHR, which may induce both cardiac hypertrophy and desensitization of β-adrenoceptor. Conversely, alterations in β-adrenoceptor activity produce a reduction in the inverse agonist activity of metoprolol.

In summary, the present study provides the first evidence of an alteration of the *in vitro* pharmacodynamic properties of metoprolol in SHR. According to our results, the sympathetic overactivity present in SHR would not only reduce the positive chronotropic effect of noradrenaline, but would also diminish the dissociation constant of metoprolol from atrial β_1-adrenoceptors and its inverse agonist activity. A significant correlation between the VW/BW ratio and the inverse agonistic potency of metoprolol was demonstrated, suggesting a possible link between cardiac hypertrophy and the reduction in the inverse agonist activity of metoprolol.

Excessive activation of cardiac β-adrenoceptor, either in response to agonist stimulation or by constitutive activity, induces deleterious effects, including cardiac hypertrophy, myocyte apoptosis, fibroblast hyperplasia and arrhythmias.[27] Conversely, inverse agonism at β_2-adrenoceptors could enhance coupling of this receptor to G_i-proteins, inhibiting hypertrophy and apoptosis of myocardial cells.[28] In addition, it was found that constitutive activity of β-adrenoceptors results in pronounced agonist stimulation of these receptors.[29,30] Therefore, inverse agonism at β-adrenoceptors could have beneficial effects in the clinical use of β-adrenoceptor antagonists. However, the blunted inverse agonist activity of beta-blockers due to receptor

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desensitization present under our experimental conditions could reflect a limitation in the therapeutic effects of this class of antihypertensive drug.

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