Beneficial Effect of the Central Nervous System β-Adrenoceptor Blockade on the Failing Heart

Andrey Gourine, Svetlana I. Bondar, K. Michael Spyer, Alexander V. Gourine

Heart failure patients are routinely given β-adrenoceptor antagonists (β-blockers), although the mechanism(s) underlying their beneficial effects is not fully resolved. It is not entirely clear how long-term application of negative inotropic compounds improves cardiac performance, slows remodeling processes, and decreases mortality. All β-blockers, which produce a beneficial effect in heart failure, have in common a high degree of lipophilicity and, therefore, have the ability to cross the blood–brain barrier. Here, we show that blockade of β-adrenoceptors directly in the brain (chronic intracerebroventricular administration of metoprolol) attenuates the progression of left ventricular remodeling in a rat model of myocardial infarction-induced heart failure. These results provide the first direct evidence that the action of certain β-blockers in the brain could contribute to their beneficial effect on the failing heart.

Mortality associated with heart failure (HF) still remains high, despite considerable achievements in medical therapy, device technology, and cardiac surgery. The use of β-adrenoceptor (β-AR) antagonists (β-blockers) pioneered in the 1970s for the treatment of arterial hypertension became one of the most important advances in HF therapy.1,2 The efficacy of 3 β-blockers, bisoprolol, carvedilol, and metoprolol, in HF treatment have been demonstrated in large placebo-controlled clinical trials.3-5 These studies have revealed a reduction in the number of deaths from worsening HF in patients treated with carvedilol and metoprolol resulting from an improvement in left ventricular (LV) function and a delayed progression of LV remodeling.3-4 In contrast, bisoprolol decreased mortality mainly by preventing sudden cardiac death,5 whereas several β-blockers (eg, bisindolol and others) have been found to be insufficiently effective to be used in HF therapy.2

A number of mechanisms explaining variable effects of β-blockers in HF patients have been proposed (including polymorphism in the genes encoding β-ARs, modulation of systemic neurohormonal activity, antagonism of the toxic actions of norepinephrine on the myocardium, favorable effects on myocardial energetics, etc).6,7 Interestingly, it appears that the β-blockers, which produce a beneficial effect in HF, all have in common a high degree of lipophilicity and, as a result, have the ability to cross the blood–brain barrier.2,8 Beneficial actions of certain lipophilic β-blockers in preventing ventricular fibrillation have already been attributed to their possible action within the brain.9 We, therefore, suggested that the favorable effects of β-blockers in HF may be attributable, at least in part, to their central nervous system (CNS) effects. To test this hypothesis, we infused metoprolol (a β-blocker widely used in HF therapy) directly into the brain and determined the effect of this treatment on the progression of LV remodeling after myocardial infarction (MI) in rats.

Materials and Methods

MI in rats was induced using a permanent coronary occlusion (CO) technique.10 Metoprolol was given into the third cerebral ventricle (ICV) 48 hours after the MI and twice daily thereafter for 6 weeks. The rats were randomized into 5 groups: (1) post-MI/artificial cerebrospinal fluid (aCSF) (animals with CO receiving ICV injections of aCSF [3 μL]); (2) post-MI/metoprolol (rats with CO receiving ICV injections of metoprolol [25 μg, 3 μL]); (3) sham/aCSF (sham-operated animals receiving ICV injections of aCSF [3 μL]); (4) sham/metoprolol (sham-operated animals receiving ICV injections of metoprolol [25 μg, 3 μL]); and (5) post-MI/metoprolol, IP (rats with CO receiving metoprolol [25 μg, 0.1 mL intraperitoneally]). Hemodynamic studies were performed 6 weeks after the surgery. Arterial pressure, heart rate (HR), LV pressure, the maximum rate of rise of LV pressure (LV dP/dt max), LV end-diastolic pressure were recorded under urethane (1.5 g/kg) anesthesia. At the end of the hemodynamic studies, LV pressure–volume relationships, LV volume, and LV infarct sizes were determined. In separate experiments in control rats, the effects of metoprolol applied ICV or into the specific hypothalamic and brain stem regions on HR were determined. Distribution of β-ARs in the brain stem regions involved in cardiovascular control was evaluated using specific antibodies. Data (means±SEM) were compared by ANOVA, followed by the Tukey–Kramer’s post hoc test to determine the main group effect. Values of P<0.05 were considered to be significant. An expanded Materials and Methods section is available in the online data supplement at http://circres.ahajournals.org.

Results and Discussion

LV remodeling is defined as progressive loss of ventricular function along with LV dilatation.11 In this study, rats from all post-MI groups were assigned into 2 groups (those with small infarcts [≤30%] and those with large infarcts [>30%] representing patients with different degrees of ischemic myocardial damage). Progression of LV remodeling and development of HF in rats is associated with elevated LV end-diastolic pressure, increased LV volume, and a shift to the right of the LV pressure–volume relationship curve (greater LV volume at any given LV pressure) (Figure 1). These data are consistent with previous observations in rats.10,12-14 In rats with small infarcts, β-AR blockade in the brain maintained nearly normal LV end-diastolic pressure (P=0.008 compared with values obtained in post-MI animals treated ICV with aCSF); LV volume to LV weight ratio (P=0.005 in comparison with post-MI/aCSF group) and prevented the right shift of the LV pressure–volume curve (Figure 1A). In rats with large infarcts, metoprolol attenuated the rise in LV end-diastolic pressure (P=0.047, compared with

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post-MI/aCSF group), although changes in LV geometry and LV pressure–volume relationships were unaffected (Figure 1B). There were no differences between experimental groups with comparable infarcts in heart weights and hemodynamic variables examined (Table I in the online data supplement).

Metoprolol given systemically in this exact dose (25 μg) was completely ineffective (Figure 1), excluding the possibility that when infused into the third cerebral ventricle, it was "leaking" out of the brain and exerting its beneficial action at some peripheral target(s). This result was somewhat predictable because the amounts of metoprolol given in this study were at least 1000 times lower than the doses required to attenuate LV remodeling in HF rats during chronic systemic administration.14 We conclude, therefore, that attenuation of LV remodeling occurs when metoprolol is acting at the sites located within the brain. The effect of central nervous β-AR blockade appears to be more prominent when ischemic myocardial damage is less severe. These results correlate well with the recent clinical data indicating that metoprolol is highly effective in patients with asymptomatic LV systolic dysfunction.4

Development of HF is associated with an increase in the activity of the sympathetic nervous system,6,15,16 which is believed to be maladaptive and detrimental, contributing to the progression of LV remodeling.6,17 Therefore, we determined whether β-AR blockade in the brain has an effect on sympathetic outflow to the heart. We analyzed metoprolol-induced changes in HR following systemic pretreatment with atenolol, a hydrophilic β1-blocker with a limited ability to cross the blood–brain barrier,8 or the M-cholinoreceptor antagonist atropine. It was found that the magnitude of the decrease in HR evoked by injection of metoprolol into the brain was not affected in conditions when parasympathetic input to the heart was interrupted by atropine (Figure 2A). In contrast, when sympathetic drive was blocked by atenolol, metoprolol had no effect on HR (Figure 2A), indicating that blockade of β1-ARs in the brain decreases sympathetic outflow to the heart.

To reveal the putative site(s) of β1-blocker action within the brain, we microinjected metoprolol into the main hypothalamic and brain stem structures involved in cardiovascular control (Figure 2B). A significant decrease in HR was observed only when metoprolol was injected into the caudal part of the medullary nucleus of the solitary tract (cNTS) (Figure 2B and 2C). Metoprolol injected into the paraventricular nucleus of the hypothalamus, anterior hypothalamus, rostral NTS, nucleus ambiguus, raphe nucleus, area postrema, dorsal motor nucleus of the vagus nerve, or rostroventrolat-

Figure 1. Blockade of β-ARs in the CNS with metoprolol attenuates the progression of LV remodeling in a rat model of MI-induced HF. A, The data from the animals with infarct sizes ≤30%. B, The data from the animals with infarct sizes >30%. Insets depict the respective infarct size (IS) (%) values for post-MI groups. Values are mean±SEM. Numbers in parentheses indicate sample sizes. #P<0.05 compared with values obtained in the respective sham-operated animals. In LV pressure–volume plots, # denotes significant (P<0.05) rightward shift of the LV pressure–volume curves in post-MI groups compared with the curves in the respective sham groups. *P<0.05 compared with values obtained in post-MI animals treated into the brain with aCSF. In the LV pressure–volume plot, * indicates significant (P<0.05) shift of the pressure–volume curve to smaller LV volumes in post-MI/metoprolol group compared with post-MI/aCSF group. LVV/LVW indicates LV volume to LV weight ratio; and LVEDP, LV end-diastolic pressure.

A

B

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eral medulla had no effect on HR. The presence of β-receptors in the cNTS was confirmed immunohistochemically (Figure 2D and 2E).

The data demonstrating the importance of the brain mechanisms in progression of HF have been reviewed recently. This study is from the same genre; it provides the first direct evidence that an action of β-blockers within the CNS could contribute to their beneficial effect on the failing heart. The decrease in sympathetic outflow to the heart following blockade of β1-receptors in the CNS seems particularly significant because detrimental sympathetic activation is believed to play an important role in the pathophysiology of HF. The cNTS is one of the possible sites of β-blockers action. Taken together, this study demonstrates the existence of previously unrecognized central nervous β-AR mechanism, the blockade of which is beneficial in HF, and may help to identify novel therapeutic strategies for HF treatment aimed at targeting sites within the brain. An expanded Discussion section is available in the online data supplement.

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None.

References


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**SUPPLEMENTARY METHODS**

**Animal model of postinfarction left ventricle (LV) remodeling.** Experiments were performed on 190 adult male Wistar rats (220-250 g) and carried out in accordance with the guidelines of the UK Animals (Scientific Procedures) Act, 1986. Myocardial infarction (MI) leading to LV remodeling and development of HF was induced using a left coronary artery occlusion technique described in detail in the literature\(^1\). All surgical procedures were performed under sterile conditions. The rats were anaesthetized (mixture of ketamine HCl [87.0 mg/kg] and xylazine HCl [13.0 mg/kg] injected intramuscularly), intubated endotracheally, and ventilated artificially. A left thoracotomy was performed to expose the heart, the pericardium was opened and the heart was exteriorized. The left anterior descending coronary artery was ligated below the left atrial appendage. The successful coronary occlusion was confirmed by pallor of the anterior wall of the left ventricle and the ST segment elevation on ECG. Then the heart was returned to the chest cavity. Sham-operated rats were prepared in the same manner but did not undergo coronary artery ligation. The lungs were reinflated, the chest incision was closed, the animal was removed from the ventilator and the endotracheal tube was removed. Then a 26-gauge guide injection cannula (Plastics One, Roanoke, VA, USA) was stereotaxically implanted into the third cerebral ventricle (stereotaxic co-ordinates: 1.0 mm caudal to bregma and 8.4 mm ventral from the surface of the skull) according to the atlas of Paxinos & Watson\(^2\). Two small screws were placed into the skull, and the cannula was secured in place by dental acrylic. The guide cannula was closed with a dummy cannula that extended from the tip of the guide cannula by ~0.2 mm. After the surgery rats were housed one per cage and were given penicillin and lidocaine.

**Experimental groups.** Postoperative mortality within 48 hours after coronary occlusion was ~40% due to sudden cardiac death. None of the surviving post-MI rats died during the remaining study period (6 weeks after coronary occlusion).
After 48 hours of recovery, the rats were randomized into five groups: (1) post-MI/aCSF – animals with permanent coronary occlusion receiving injections of artificial cerebrospinal fluid (aCSF, 3 µl) into the third cerebral ventricle; (2) post-MI/metoprolol – rats with permanent coronary occlusion receiving injections of metoprolol (25 µg, 3 µl) into the third cerebral ventricle; (3) sham/aCSF – sham-operated animals receiving injections of aCSF (3 µl) into the third cerebral ventricle; (4) sham/metoprolol – sham-operated animals receiving injections of metoprolol (25 µg, 3 µl) into the third cerebral ventricle; (5) post-MI/metoprolol, i.p. – rats with permanent coronary occlusion receiving metoprolol (25 µg, 0.1 ml) intraperitoneally.

**Treatments.** Metoprolol was given into the third cerebral ventricle 48 hours after myocardial infarction and twice daily thereafter for 6 weeks, i.e. within a realistic clinical timeframe. The dose of metoprolol had been chosen on the basis of a pilot study using 61 conscious freely-moving rats. Metoprolol was given into the third cerebral ventricle in doses 5, 25, 50 and 100 µg and was found to decrease the heart rate after 15 min by 7±12 (n=11, P>0.05), 39±6 (n=13, P<0.05), 47±10 (n=16, P<0.05) and 69±6 (n=10, P<0.05) beats/min, respectively. Therefore, metoprolol dose of 25 µg had been chosen as a threshold amount of the β-blocker required to induce a modest decrease in heart rate after an acute intracerebroventricular injection.

Microinjections (volume 3 µl) into the third cerebral ventricle were made over a period of 1-2 min using an internal injection cannula connected to PE-20 tubing attached to a 10 µl syringe. The injection cannula was removed 2-3 min after the injection. In the post-MI/metoprolol, i.p. group metoprolol was given i.p. in a volume of 0.1 ml.

**Hemodynamic studies** were performed 6 weeks after MI or sham surgery, i.e. when HF is fully developed in the MI rats. Urethane (1.5 g/kg, i.p.) was used as an anesthetic of choice because it does not appear to suppress cardiac vagal tone. Adequate anesthesia was ensured by maintaining stable levels of arterial blood
pressure and heart rate and monitored by the absence of a withdrawal response to a paw pinch. The femoral artery and the right jugular vein were cannulated for measurement of mean arterial pressure (MAP) and administration of drugs, respectively. The trachea was cannulated and the animal was ventilated artificially with room air using a positive pressure ventilator (Harvard rodent ventilator, model 683) with a tidal volume of ~2 ml at a respiratory frequency similar to spontaneous frequency (~60 strokes/min). To evaluate left ventricular (LV) contractile function, LV pressure was recorded using a 2-Fr Millar SPR-407 microtip catheter (Millar Instruments, Houston, TX) introduced into the LV chamber via the right carotid artery. MAP, LV pressure, the maximum rate of rise of LV pressure (LV dP/dtmax), LV end-diastolic pressure (LVEDP) and ECG were processed via a 1401 CED interface, recorded and analyzed using Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

**LV pressure-volume relations, LV volume and LV infarct size.** LV pressure-volume curves were obtained using a double-lumen catheter, by a previously described method. At the end of the hemodynamic studies the heart was arrested by potassium chloride and a double-lumen catheter (PE-50 inside PE-200) was inserted into the LV via the ascending aorta. The right ventricle was incised and atrioventricular groove was ligated, and isotonic saline was infused at a rate of 0.70 ml/min via one lumen while intraventricular pressure was continuously recorded through the other lumen from negative pressure to 30 mmHg. At least three measurements of the pressure-volume curves were obtained. Then the atria and right ventricle were excised, LV was weighted and 10% phosphate-buffered formalin was infused under constant pressure (7.5 mmHg) continuously for 24 hours into the LV chamber. After fixation, LV volume was measured and the base of the heart was excised at the level of the atrioventricular groove. The LV cavity was blotted dry and then filled with saline by 1 ml syringe. For each heart three measurements of LV volume were taken and averaged.

The extent of myocardial infarction was determined as described in detail previously. The LV was cut from the apex to the base into four transverse slices.
of identical thickness (1.5 mm). In each slice, the length of the scar and noninfarcted muscle for endocardial and epicardial surfaces were determined by computerized planimetry. The ratio of the length of the scar and surface circumferences defined the infarct size for endo- and epicardial surfaces, respectively. Infarct size (in %) was calculated as an average of infarcted endo- and epicardial surfaces.

**Effect of intracerebroventricular metoprolol on cardiac sympathetic tone.** In a separate set of experiments in rats (n=30), the relative contribution of parasympathetic activation and sympathetic withdrawal in mediating decreases in heart rate following metoprolol infusion into the brain was investigated. We analyzed changes in heart rate after metoprolol injection into the brain in basal conditions and following systemic administration of atenolol (a hydrophilic $\beta_1$-blocker with a very limited ability to cross the blood-brain barrier) or M-cholinoreceptor antagonist atropine. In anaesthetized (urethane, 1.5 g/kg) rats metoprolol (25 µg) was injected into the third cerebral ventricle 5 min after the intravenous injections of either saline, atenolol (1 mg/kg) or atropine (2 mg/kg). The effect of this treatment on heart rate was determined.

**Effects of metoprolol microinjected into discrete central nervous system locations.** To determine the possible site(s) of β-blockers action within the brain we microinjected metoprolol into the hypothalamic (paraventricular nucleus) and brainstem (nucleus of the solitary tract, nucleus ambiguus, area postrema, raphé nucleus, rostral ventrolateral medulla) structures which are involved in cardiovascular control and determined the level of sympathetic activity.

The animal was prepared as described above (see Hemodynamic studies) and was placed in a stereotaxic frame. A three-barreled micropipette (tip size 15-20 µm) was placed in the area of interest using stereotaxic co-ordinates. The barrels of the micropipette contained aCSF (pH 7.4) metoprolol (dissolved in aCSF) and Pontamine Sky Blue dye (2% in 0.2 M sodium acetate). The injections (40 nl) were made using...
pressure over a period of 5-10 s and were monitored using a dissecting microscope with a calibrated micrometre disk. The sites of microinjections were marked by pressure injection of Pontamine Sky Blue dye, identified histologically and mapped using a stereotaxic atlas\textsuperscript{2}.

**\( \beta_1 \)-adrenoreceptor immunohistochemistry.** The rats (n=6) were deeply anaesthetized with pentobarbitone sodium (100 mg/kg, ip) and perfused transcardially with 400 ml of heparinised saline followed by 400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and post-fixed in the same fixative for 12 hours at 4°C. Coronal sections (50 \( \mu \)m) of the medulla oblongata were cut and collected into phosphate-buffered saline (PBS; pH 7.4). Sections were incubated for 2 h at room temperature in a preblock solution (10% normal goat serum, 0.1% Triton X-100 in PBS) followed by 3 x 5 min washes in PBS. Sections were then incubated for 12 h at 4°C with the primary antibodies: rabbit-anti-rat \( \beta_1 \)-adrenoreceptor (1:500; Santa Cruz Biotechnology, USA). Following 3 x 10 min washes in PBS, the sections were incubated for 2 h in goat anti-rabbit IgG conjugated to Cy3 (1:500 dilution; Jackson ImmunoResearch Laboratories, USA). Sections were then washed for 3 x 10 min in PBS, air dried onto glass slides, mounted under a coverslip using VectaMount (Vector Labs) and viewed under a Zeiss Axioplan fluorescent microscope (Jena, Germany) using the appropriate filter set.

**Statistical Analysis.** Data are reported as mean ± s.e.m. Data were compared by ANOVA followed by the Tukey-Kramer’s post hoc test to determine the main group effect. Values of \( P<0.05 \) were considered to be significant.
SUPPLEMENTARY DISCUSSION

The data obtained in the present study demonstrate the existence of a previously unrecognized central nervous mechanism which may be responsible, at least in part, for the beneficial effect of β-adrenoreceptor (β-AR) blockade on the failing heart. Chronic administration of the β₁-blocker metoprolol directly into the brain has been found to attenuate the progression of left ventricular (LV) remodeling in a rat model of myocardial infarction-induced heart failure (HF). This action of metoprolol decreases sympathetic outflow to the heart. The results of the present study also suggest that the caudal part of the medullary nucleus of the solitary tract is a possible site of β-blockers action within the CNS.

LV remodeling is defined as a progressive loss of ventricular function together with LV dilatation⁴. The rat model of LV remodeling and HF used in this study closely resembles the manifestation of HF in post-myocardial infarction (MI) patients and is associated with an elevated LV end-diastolic pressure (LVEDP), increased LV volume and a shift to the right of the LV pressure-volume relationship curve. These data are consistent with previous observations in rats¹,³,⁵. In rats with small infarcts (≤30%) β-AR blockade in the brain had a striking beneficial effect – it resulted in a nearly normal LVEDP, LV volume to LV weight ratio and completely prevented the shift of the LV pressure-volume curve to the right. In rats with large infarcts metoprolol attenuated the rise in LVEDP without affecting changes in LV geometry and LV pressure-volume relationships. These results correlate well with the clinical data indicating that metoprolol may be also effective in patients with asymptomatic LV systolic dysfunction⁶ as well as in patients with advanced HF⁷,⁸

Metoprolol, blood brain barrier and effective doses

Metoprolol is a lipophilic compound and readily crosses the blood brain barrier⁹,¹⁰. When infused into the brain it may “leak” into the systemic circulation,
probably as easy as it penetrates into the brain after peripheral administration. In a control experiment metoprolol was found to be completely ineffective when it was given systemically in the same dose (25 µg twice daily) which had a strong beneficial effect when infused into the brain. This result was somewhat predictable since the amounts of metoprolol given in this study were at least 1000 times lower than the doses previously shown to improve LV function and attenuate LV chamber dilatation in post-MI rats after chronic systemic administration\textsuperscript{11,12}. Thus, it is highly unlikely that in our experiments metoprolol infused into the third cerebral ventricle was “leaking” out of the brain and exerting its beneficial effect via action at some peripheral target(s). We conclude, therefore, that attenuation of the progression of LV remodeling occurs when metoprolol is acting at the sites located within the CNS.

Although, the data obtained indicate the existence of a central nervous β-AR mechanism, blockade of which is beneficial in HF, it may be argued that the concentration of metoprolol in the cerebrospinal fluid (CSF) and the brain following i.c.v. infusion in our experiments by far exceeds the levels achieved when the β-blocker is given systemically\textsuperscript{11,12}. This possibility of course cannot be completely excluded without direct comparison of metoprolol concentrations in the brain tissue following its peripheral or intracerebroventricular (i.c.v.) injections. We believe, however, that the doses of metoprolol infused into the brain in our experiments were not exceedingly high. Indeed, as mentioned above the amounts we injected into the brain were at least 1000 times lower than the amounts required to improve LV function in post-MI rats during chronic systemic administration\textsuperscript{11,12}. Furthermore, a recent study in rabbits demonstrated that when metoprolol is infused systemically - CSF concentration of the drug is almost identical to its plasma level\textsuperscript{9}. It is clear that the brain is very well exposed to the changes of plasma concentrations of metoprolol achieved when this β-blocker is given systemically.

It is also rather difficult to compare the doses of metoprolol used in this study with that of metoprolol prescribed clinically. There is no general consensus regarding
whether or not patients need to take a specific target β-blocker dose in order to receive benefit. The results of MERIT-HF trial indicate that reduction of total mortality in HF was similar in patient groups receiving high- and low- doses of metoprolol\textsuperscript{13}. It was concluded that in both groups similar level of β-AR blockade was achieved as judged by similar reduction in heart rate. These results have led to an individualized dose-titration regime which is guided by patient tolerability and the magnitude of heart rate reduction. Thus, clinically metoprolol (and other β-blockers) are used in incremental doses, while in our study as well as in other experimental studies it was given in constant doses. In the previous experimental studies\textsuperscript{11,12} metoprolol was administered in amounts, required to reduce the heart rate by about 15-20% from its basal level. The same approach was also used in the current study.

*Heart failure, autonomic control of the heart and metoprolol actions*

Development of HF is associated with a decrease in cardiac vagal tone\textsuperscript{14} and an increase in the activity of the sympathetic nervous system\textsuperscript{15,16}, which is generally believed to be maladaptive and detrimental, contributing to the progression of LV remodeling\textsuperscript{16-18}. Sanderson et al.\textsuperscript{19} demonstrated superior effects of metoprolol over hydrophilic vasodilating β-blocker celiprolol in restoring baroreflex gain and vagal tone in patients with chronic HF. Similarly, recent evidence by Ablad et al (2007) obtained in anaesthetized rabbits suggested that β-blockers are acting centrally to increase cardiac vagal tone. In contrast, there are data obtained in normal human subjects\textsuperscript{20,21} and in post-MI patients\textsuperscript{22,23} indicating that the effects of β-blockers on vagal tone are likely to be due to their peripheral actions.

Because of this controversy, in the present study the effects of β-AR blockade in the brain on sympathetic and vagal outflows to the heart have been determined by comparing metoprolol-induced changes in heart rate following systemic pre-treatment with atenolol – a hydrophilic β\textsubscript{1}-blocker with a very limited ability to cross the blood-brain barrier\textsuperscript{10}, and the M-cholinoreceptor antagonist atropine. It was found that the magnitude and the time course of the decrease in heart rate evoked
by injection of metoprolol into the brain is not at all affected when the parasympathetic input to the heart is interrupted by atropine. In contrast, when sympathetic drive was blocked by atenolol, metoprolol had no effect on heart rate.

It could be argued that systemic treatment with atenolol reduces basal heart rate to a certain level and further decrease in response to i.c.v. metoprolol can no longer occur. Indeed, the average resting heart rate after atenolol was ~340 bpm, representing an approx 20% reduction from the baseline. Atenolol was given in a dose of 1 mg/kg –which is generally believed to be sufficient to block most of the sympathetic influences to the heart. It is not, however, a maximum effect as heart rate in rats can be lowered further by a number of different pharmacological agents. For example, sinus node inhibitors (like zatebradine and ivabradine) reduce heart rate by 30% without major effects on cardiac output and contractility\textsuperscript{24,25}. Furthermore, electrical stimulation of the vagus nerve can even arrest the heart in diastole, hence, if metoprolol had any central stimulatory effect on parasympathetic outflow we should have detected a further decrease in heart rate after i.c.v. metoprolol treatment. However, this was not the case.

Taken together the data obtained in the present study strongly suggest that metoprolol actions in the CNS decrease sympathetic outflow to the heart, while having no obvious effect on cardiac vagal tone.

These experiments were conducted in control rats. Of course, the effects of chronic i.c.v. metoprolol treatment in post-MI animals is impossible to compare with the effects of this β-blocker given acutely in anesthetized, but otherwise healthy rats. However, in the pilot study (n=4) we found, that the effect of metoprolol on heart rate in conscious post-MI animals (three days after coronary artery occlusion) was similar to that observed in sham-operated animals. These results are in accord with the data available in the literature indicating that the magnitude of heart rate reduction (around 20% from the baseline) induced by systemic metoprolol treatment in post-MI rats varied to a minimal degree during 12 weeks treatment.
period. Therefore, there were no obvious reasons to conduct these experiments in post-MI animals.

**Putative site of action**

To reveal the putative site(s) of β-blocker action within the brain, metoprolol was microinjected into the main hypothalamic and brainstem structures involved in cardiovascular control. A significant decrease in HR was observed only when metoprolol was injected into the caudal part of the medullary nucleus of the solitary tract (cNTS).

However, direct evidence of cNTS role as the site of β-blocker beneficial action on the failing heart remains to be obtained. Specific approaches to provide unequivocal evidence that cNTS is indeed the site of β-blockers action within the brain are not available at the moment. Applications of metoprolol-releasing pellets on the dorsal hindbrain surface or intra-cisternal infusions are not superior to microinjections into the 3rd cerebral ventricle as the rate of diffusion and the spread of the drug in the brainstem are impossible to control with either of these methods. The use of siRNAs to suppress the expression of β-ARs in the NTS was also considered. Obviously, to prevent siRNA effect from spreading to unspecified areas of the brain, this can only be done by viral expression of shRNA- or miRNA-based constructs. This, however, is not a method currently established and validated for the brainstem. In addition, existing siRNAs developed to silence β₁-ARs are all imperfect – their systemic effects on heart rate and contractility are drastically different from those induced by β-blockers.

Although indirect, the data obtained in the current study suggest that cNTS is indeed the most likely site of the central nervous β-blocker’s action in heart failure. NTS is very superficial and considering the direction of CSF flow there is very little doubt that metoprolol rapidly reaches this brainstem structure after injection into the third cerebral ventricle. Most importantly, it appears, that among major hypothalamic and brainstem structures involved in cardiovascular control cNTS is
the only site in which metoprolol injection has an effect on cardiovascular activity. In addition, we demonstrate in this report the abundance of $\beta_1$-ARs expression in the cNTS.

NTS is the major brainstem nucleus which receives and integrates information from the peripheral chemoreceptors, cardiovascular and pulmonary afferents$^{29-31}$. cNTS in particular receives inputs from the carotid baro- and chemoreceptors as well as from the aortic baroreceptors$^{29-31}$. The importance of NTS mechanisms in the development of arterial hypertension has been recently demonstrated$^{32}$. The exact mechanisms of how $\beta$-AR activity in the NTS contributes to the development of HF remains to be established.

Central nervous system and the failing heart

The data indicating the importance of brain mechanisms in the progression of HF have been reviewed recently$^{18}$. The chronic central infusion of either AT$_1$-receptor antagonist losartan, aldosterone receptor antagonist spironolactone, Fab fragments to block “ouabain”, or central gene transfer of interleukin-10 to reduce hypothalamic inflammation - all to a different extent reduced LV remodeling and cardiac dysfunction in rats following MI$^{33-36}$. Wang et al.$^5$ described attenuation of LV remodeling, improved LV function and lower sympathetic activity after MI in transgenic rats deficient in brain angiotensinogen. Taken together these data suggest that the brain renin-angiotensin-aldosterone system and upregulation of brain cytokines may contribute to the development of LV dysfunction following MI. Here using the same rat model of MI-induced HF we demonstrated that the blockade of $\beta_1$-ARs in the brain is having a similar beneficial effect. Whether brain $\beta$-AR mechanism interacts with renin-angiotensin-aldosterone and cytokine systems in contributing to the development of LV dysfunction in HF remains to be determined.
Conclusion

The data obtained strongly indicate the existence of the central nervous β-AR mechanism, blockade of which is beneficial in HF. By extension, these results provide the first direct evidence that an action of β-blockers within the CNS could underlie their beneficial effect on the failing heart. This effect of central nervous β-AR blockade appears to be more prominent when ischemic myocardial damage is less severe. The decrease in sympathetic outflow to the heart following blockade of β₁-ARs in the CNS seems particularly significant since detrimental sympathetic activation is believed to play an important role in the pathophysiology of HF. The caudal NTS is one of the possible sites of β-blockers action. Taken together, this study may further facilitate the development of novel therapeutic strategies for HF treatment aimed at targeting sites within the CNS.
References


23. Tuininga YS, Crijns HJ, Brouwer J, van den Berg MP, Man i, V, Mulder G, Lie KI. Evaluation of importance of central effects of atenolol and metoprolol measured by heart rate variability during mental performance tasks,


**Supplementary Table.** Ventricular weight/body weight ratios and baseline hemodynamics in rats treated with Metoprolol or artificial cerebrospinal fluid for 6 weeks after myocardial infarction or sham surgery.

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated groups</th>
<th>Post-Myocardial Infarction groups</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(≤30%)</td>
<td>(&gt;30%)</td>
<td>(i.p.)</td>
<td>aCSF</td>
<td>Metoprolol</td>
<td>aCSF</td>
<td>Metoprolol</td>
<td>Metoprolol</td>
<td>aCSF</td>
</tr>
<tr>
<td>Infarct Size, % of LV</td>
<td></td>
<td></td>
<td>24±1</td>
<td>24±1</td>
<td>23±2</td>
<td>38±2</td>
<td>39±1</td>
<td>37±2</td>
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<tr>
<td>LV weight/ BW, g/kg</td>
<td>1.81±0.05</td>
<td>1.89±0.05</td>
<td>2.04±0.07</td>
<td>1.99±0.09</td>
<td>1.99±0.08</td>
<td>2.00±0.07</td>
<td>2.01±0.05</td>
<td>1.98±0.06</td>
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<tr>
<td>RV weight/ BW, g/kg</td>
<td>0.50±0.03</td>
<td>0.53±0.01</td>
<td>0.56±0.03</td>
<td>0.6±0.03</td>
<td>0.55±0.02</td>
<td>1.03±0.14*</td>
<td>0.8±0.07®</td>
<td>0.95±0.12®</td>
<td></td>
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</tr>
<tr>
<td>MAP, mmHg</td>
<td>106±5</td>
<td>97±4</td>
<td>93±5</td>
<td>97±7</td>
<td>99±11</td>
<td>92±7</td>
<td>86±3</td>
<td>91±5</td>
<td></td>
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</tr>
<tr>
<td>Heart rate, bpm</td>
<td>452±15</td>
<td>448±8</td>
<td>422±17</td>
<td>421±16</td>
<td>424±14</td>
<td>439±5</td>
<td>432±7</td>
<td>433±12</td>
<td></td>
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<tr>
<td>LVSP, mmHg</td>
<td>129±4</td>
<td>127±5</td>
<td>120±4</td>
<td>118±5</td>
<td>123±6</td>
<td>115±7</td>
<td>113±5</td>
<td>116±9</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LV dP/dt max, mmHg/s</td>
<td>7085±331</td>
<td>6555±502</td>
<td>6199±664</td>
<td>5965±630</td>
<td>6041±804</td>
<td>5463±417*</td>
<td>5209±641</td>
<td>5027±235</td>
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</tr>
</tbody>
</table>

Values are means ± s.e.m. (n=8-12 animals per group). aCSF, artificial cerebrospinal fluid. LV, left ventricle. RV, right ventricle. BW, body weight. MAP, mean arterial pressure. LVSP, LV systolic pressure. LV dP/dt max, maximum rate of LV pressure rise. Data were compared by ANOVA followed by the Tukey-Kramer’s post hoc test to determine the main group effect. *P<0.05 compared to values obtained in Sham-operated animals treated into the brain with aCSF. ®P<0.05 compared to values obtained in Sham-operated animals treated into the brain with Metoprolol.