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Involvement of nitric oxide in both central and peripheral haemodynamic effect of D/L-nebivolol and its enantiomers in rats

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Abstract

The cardiovascular profile of the racemate D/L-nebivolol and its enantiomers administered by intravenous (i.v.) or by intracerebroventricular (i.c.v.) route was investigated in anaesthetized normotensive rats. D/L-Nebivolol (0.1–0.5 mg/kg) induced a dose-related reduction in blood pressure when administered by i.c.v. route. These hypotensive effects were more marked as compared to those achieved by peripheral administration of D/L-nebivolol (0.1–1 mg/kg i.v.). Both enantiomers contributed to the hypotensive effect of D/L-nebivolol by i.c.v. route, while the effects of the drug on blood pressure by i.v. route were due to the D-enantiomer. The bradycardic effect of the racemic form given i.v. was dose-related and, at the highest dose (1 mg/kg), was more pronounced as compared to i.c.v. route. D-Nebivolol was responsible for chronotropic effects by both the i.v. and i.c.v. route, although by i.c.v. route L-nebivolol also induced a reduction in heart rate. The nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) administered at 5 mg/kg i.v. bolus+0.1 mg/kg/min infusion or at 2.5 mg/kg i.c.v. counteracted the effects of D/L-nebivolol (either 1 mg/kg i.v. or 0.5 mg/kg i.c.v.) on blood pressure, while it did not inhibit the cardiovascular changes induced by isoprenaline (300 ng/kg i.v.) or calcitonin gene-related peptide (CGRP; 400 ng/kg i.v.). In addition, i.c.v. effects of D/L-nebivolol on blood pressure and heart rate were not affected by pre-treatment with atropine (2 mg/kg i.v.). The present findings demonstrate that D/L-nebivolol produced haemodynamic changes following both peripheral and central administration; these latter findings are mainly due to its L-enantiomer and these effects involve the L-arginine/nitric oxide pathway.

Keywords: β-Adrenoceptor; β-Adrenoceptor antagonist; Hypertension; D/L-Nebivolol; NO (nitric oxide); Vasodilation

1. Introduction

D/L-Nebivolol is a lipophilic, third-generation β-adrenoceptor antagonist, devoid of intrinsic sympathomimetic activity (Janssens et al., 1989; Brixius et al., 2001), which has been shown to possess β_1-adrenoceptor selectivity and vasodilation properties (Van de Water et al., 1988; Bundkirchen et al., 2003). The compound is a racemate mixture of two enantiomers (D and L) and the D-isomer of D/L-nebivolol is considered responsible for highly selective antagonism on β_1-adrenoceptor. D/L-Nebivolol has been also shown to possess vasodilatory effects involving the nitric oxide (NO) dependent pathway (Gao et al., 1991; Ignarro, 2004). Recent in vitro studies demonstrated that in certain vascular districts (particularly in small diameter, non-conduit vessels, and in platelets), D/L-nebivolol can stimulate an increase of NO, which becomes available at the smooth muscle layers and induces vasorelaxation (Altwegg et al., 2000; Falciani et al., 2001). This ability to reduce peripheral vascular resistance with NO involvement has been confirmed in clinical studies in volunteers and hypertensive patients (Bowman et al., 1994; Cockroft et al., 1995; Kakoki et al., 1999; Tzemos et al., 2001).

Past studies have shown that D/L-nebivolol is able to exert these peculiar haemodynamic effects by i.v. (Gry-

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glewski et al., 2001) or i.c.v. (Midol-Monnet et al., 1991) route; however, the mechanisms of this effect have not been directly investigated.

Therefore, the aim of the present study is to verify if NO-dependent pathways are involved in haemodynamic effects of D/L-nebivolol when administered by central or peripheral route and how the D- and L-enantiomers contribute to these effects.

2. Materials and methods

2.1. Animals

Wistar rats (Harlan, Corezzana, Bergamo, Italy), approximately 9–10 weeks old were used. Animals were housed in polycarbonate cage under continuously monitored environmental conditions. Drinking water and a specific powdered diet (Altromin MT, Rieper, Bolzano, Italy) were supplied ad libitum. Environmental conditions, as well as the procedures for housing and handling the animals, were in compliance with EU and Italian Guidelines (D.L. no. 116/92) for Laboratory Animal Welfare. Experimental procedures were also examined and approved by internal ethical committee for animal welfare.

2.2. Animal preparation and haemodynamic measurements

Animals were anaesthetized with 0.4 ml/kg of Hypnorm (fentanyl citrate, 0.315 mg/ml and fluanisone 10 mg/ml) and 60 mg/kg of pentobarbital sodium, both given by intraperitoneal (i.p.) route. After the induction of anaesthesia, animals were placed on a homeothermic blanket system to maintain the body temperature at 37 °C; anaesthesia was administered using pentobarbital sodium (5 mg/ml; flow rate 0.5–3 ml/h) by i.v. infusion. The trachea was exposed, and a polyethylene cannula inserted to allow good ventilation. Two catheters (Angiocath 24 G; Becton Dickinson, Sandy, Utah, USA) were inserted into tail veins for infusion of additional pentobarbital sodium and for administration of test substances. A stainless-steel guide cannula was implanted into the right cerebral ventricle. The coordinates used to implant the cannula were derived from the atlas of the rat brain (Pellegrino et al., 1986) and were chosen as follows: 0.5–0.7 mm posterior to the bregma, 1.5–1.7 mm lateral to the midline and 3.5–4 mm below the skull surface. The guide cannula was fixed on the skull surface with dental cement. The correct position of the guide cannula into the lateral cerebral ventricle was confirmed by the spontaneous flow of cerebrospinal fluid from the tip of the cannula and by injection, at the end of the experiment, of blue Evans dye through the cannula with postmortem examination of the brain. Alternatively, at the end of the experiment, i.c.v. injection of angiotensin II (2 μg/animal in 5 μl) was performed and subsequent arterial pressure response verified.

Blood pressure was measured by means of a polyethylene cannula (PE 50) filled with heparinized saline (25 IU/ml) inserted into the left femoral artery. The cannula was connected to a transducer (Transpack, Abbott, North Chicago, USA) and the signal was amplified by means of a BM 614/2 amplifier (Biomedica Mangoni, Pisa, Italy).

Heart rate was obtained from the blood pressure signal by means of an acquisition data system (Ponemah, Gould G.N. Sistemi, Milano, Italy); both signals were continuously recorded.

2.3. Study design

After completion of surgical preparation, animals were subjected to treatment as indicated below:

2.3.1. Protocol 1

Animals received D/L-nebivolol at 0.1, 0.5 and 1 mg/kg by i.v. injection. Control animals received vehicle (β-cyclodextrin 15% in sterile water) at the same dose volume (1 ml/kg). Blood pressure and heart rate were monitored for 1 h after D/L-nebivolol administration. Cardiovascular effects of D- and L-nebivolol, administered at 0.5 mg/kg by i.v. route, were investigated. The effects of i.v. treatment with D/L-nebivolol at 1 mg/kg and D- and L-nebivolol at 0.5 mg/kg against adrenoceptors stimulation with isoprenaline at 300 ng/kg (1 ml/kg) by i.v. route were also evaluated.

2.3.2. Protocol 2

Animals received, by i.c.v. injection, D/L-nebivolol at 0.1, 0.25 and 0.5 mg/kg in 10 μl; control animals received vehicle (β-cyclodextrin 15% in sterile water) at the same dose volume. Blood pressure and heart rate were monitored for 1 h after D/L-nebivolol administration. Centrally mediated cardiovascular effects of D- and L-nebivolol at 0.25 mg/kg were also investigated. Effects of D/L-nebivolol at 0.1 mg/kg i.c.v. against adrenoceptors stimulation with isoprenaline at 300 ng/kg (1 ml/kg) by i.v. route were also evaluated.

2.3.3. Protocol 3

In addition, the effects of D/L-nebivolol at 1 mg/kg i.v. and 0.5 mg/kg i.c.v. were investigated in rat pre-treated with L-NAME i.v. (5 mg/kg+0.1 mg/kg/min) or i.c.v. (2.5 mg/kg in 8–10 μl). Control animals received vehicle (β-cyclodextrin 15% in sterile water) at the same dose volume. Blood pressure and heart rate were monitored for 1 h after D/L-nebivolol administration.

To verify the selectivity of L-NAME-induced hypertension, additional experiments were performed by bolus i.v. injection of isoprenaline (300 ng/kg) and CGRP (400 ng/kg) before and after L-NAME treatment. The treatment with L-NAME was performed 15 min after the first injection of isoprenaline or CGRP when their effects on HR and BP returned to the pre-dose level. The second treatment with isoprenaline or CGRP was given at the peak of L-NAME-induced hypertension approximately 20 min after its bolus injection.

In addition, to exclude the involvement of the parasympathetic nervous system in the haemodynamic effects of D/L-nebivolol, further experiments were performed with D/L-nebivolol at 0.5 mg/kg i.c.v. in rats pre-treated with atropine at 2 mg/kg i.v.

2.4. Drugs

D/L-Nebivolol, batches nn. 94361 and 40578, and D- and L-nebivolol, batches nn. R085547 and R085548, were synthesized at the chemical department of Berlin Chemie (Berlin, Germany). L-NAME, isoprenaline, CGRP, atropine and β-cyclodextrin came from Sigma Chem. (Milano, Italy). Hypnorm came from Janssen-Cilag, Saunderton, Buckinghamshire, UK. Pentobarbital sodium came from Sigma Chem., St. Louis, MO, USA.

2.5. Statistical analysis

All the data presented in the text, tables and figures are means±standard error of the mean (S.E.M.). Statistical comparisons were performed using a one-way analysis of variance followed by Bonferroni's *T*-test.

3. Results

3.1. Effects of intravenous treatment with D/L-, D- and L-nebivolol on heart rate and arterial blood pressure

I.v. administration of D/L-nebivolol at 0.1–1 mg/kg induced a dose-related and significant reduction in blood pressure (Fig. 1A) and heart rate (Fig. 1B).

I.v. D/L-nebivolol treatment at the lower dose level (0.1 mg/kg) did not induce any change in arterial blood pressure. A slight decrease in this parameter became evident at 0.5 mg/kg, and a significant reduction in arterial blood pressure was recorded at a dose of 1 mg/kg (Fig. 1A). The decrease in blood pressure (basal value 109±3 mm Hg) was slow in onset and long-lasting, continuing for 1 h (Fig. 1A) and more (data not shown).

The bradycardic effect of D/L-nebivolol (basal value 403±14 beats/min) started immediately after dosing, reached the maximum within 10–15 min, and continued up to 1 h after treatment (Fig. 1B).

Heart rate increase induced by isoprenaline (300 ng/kg i.v.) was almost completely abolished 15 min after administration of 1 mg/kg of D/L-nebivolol and this inhibiting effect (due to the β_1-blocking properties of the drug) was still significant 1 h after treatment (Fig. 2).

The i.v. injection of the enantiomers showed that 0.5 mg/kg of D-nebivolol induced a significant reduction in heart rate (basal value 388±12 beats/min) and blood pressure (basal value 105±1) that reached a maximum within 10–15 min after treatment (Fig. 3A and B). Haemodynamic effects exerted by D-nebivolol at 0.5 mg/kg were comparable to those induced by the racemate of nebivolol administered at 1 mg/kg, while L-nebivolol at 0.5 mg/kg did not produce any significant change in heart rate (basal value 387±12 beats/min) or arterial blood pressure (Fig. 3A and B; basal value 104±5 mm Hg). In keeping with these findings, the heart rate increase induced by isoprenaline was significantly prevented by 0.5 mg/kg i.v. of D-nebivolol, but not by the same dose of L-nebivolol (Fig. 2). These results suggest that haemodynamic effects of racemic nebivolol administered i.v. are almost completely due to its D-enantiomer.

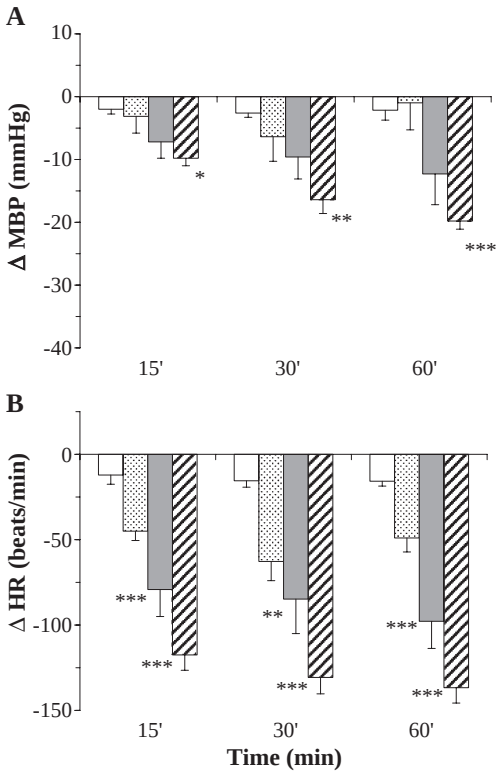

Fig. 1. Dose-dependent effect of i.v. injection of D/L-nebivolol on mean blood pressure (A) and on heart rate (B) in normotensive rats. Effects of vehicle (open bar), and of D/L-nebivolol at 0.1 mg/kg (dotted bar), 0.5 mg/kg (grey bar) and 1 mg/kg (hunched bar). Data reported are mean differences vs. basal values of 6–8 rats. **P*<0.05; ***P*<0.01; ****P*<0.001 vs. control group.

3.2. Effects of intracerebroventricular treatment with D-, L- and D/L-nebivolol on heart rate and arterial blood pressure

I.c.v. administration of D/L-nebivolol (0.1, 0.25 and 0.5 mg/kg; basal value 115±4; 120±5; 114±4 mm Hg, respectively) induced a dose-related reduction in blood pressure (Fig. 4A). This reduction began few minutes after treatment and reached a maximum within 15–20 min (Fig. 4A). D/L-Nebivolol-induced decrease in blood pressure reached the significant effect at the i.c.v. dose of 0.1 mg/kg. It should be noted that the same dose given by i.v. route did not induce any significant change in blood pressure and, a 10 times greater dose (1 mg/kg) was necessary using the i.v.

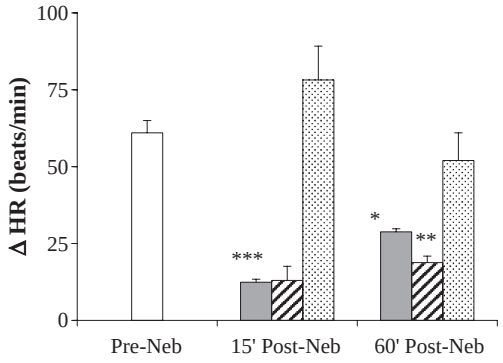

Fig. 2. Effects of i.v. injections of D/L-, D- and L-nebivolol on isoprenaline-induced heart rate increase. Effect of isoprenaline (300 ng/kg) before nebivolol (open bar), after 1 mg/kg of D/L-nebivolol (grey bar), after 0.5 mg/kg of D-nebivolol (hunched bar) or L-nebivolol (dotted bar). Data reported are mean differences vs. basal value of 5–8 rats. *P<0.05; **P<0.01; ***P<0.001 vs. basal value.

administration (Fig. 1A) in order to get a hypotensive effect similar to that induced with D/L-nebivolol at 0.1 mg/kg i.c.v.

Unlike to the effect on blood pressure, the bradycardic effect (Fig. 4) of i.c.v. D/L-nebivolol (0.1, 0.25 and 0.5 mg/kg; basal value 374±17; 387±7; 358±14 beats/min, respectively) was comparable to that elicited by the same dose of D/L-nebivolol (0.1 and 0.5 mg/kg) injected into the tail vein (Fig. 1B).

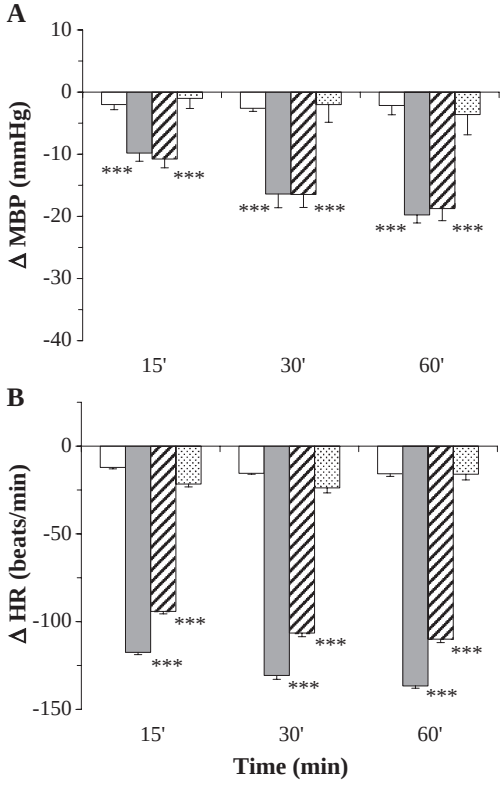

Fig. 3. Effects of i.v. injections of D/L-, D- and L-nebivolol on mean blood pressure (A) and on heart rate (B). Effects of vehicle (open bar), D/L-nebivolol at 1 mg/kg (grey bar), D-nebivolol (hunched bar) and L-nebivolol (dotted bar) both at 0.5 mg/kg. Data reported are mean differences vs. basal value of 5–8 rats. ***P<0.001 vs. control group.

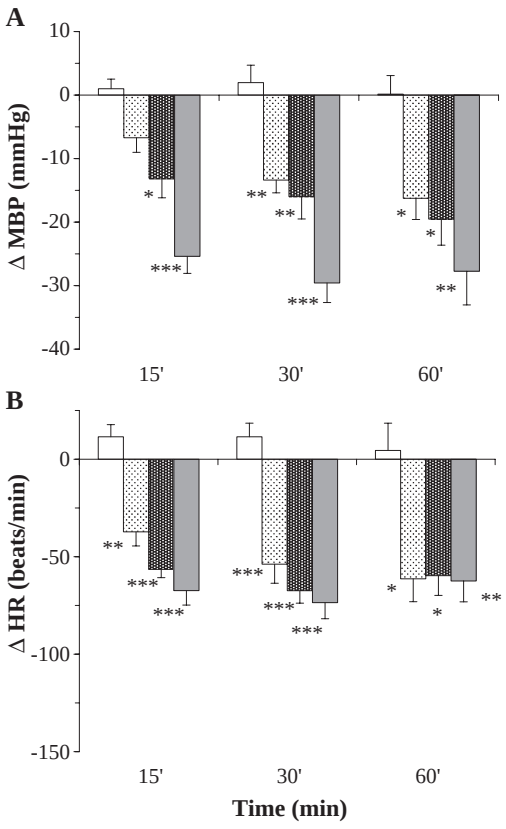

Fig. 4. Dose-dependent effect of i.c.v. D/L-nebivolol on mean blood pressure (A) and on heart rate (B) in normotensive rats. Effects of vehicle (open bar), D/L-nebivolol at 0.1 (dotted bar), 0.25 (cross-hunched bar) or 0.5 mg/kg (grey bar). Data reported are mean differences vs. basal values of 6–8 rats. *P<0.05; **P<0.01; ***P<0.001 vs. control group.

I.c.v injection of D- and L-nebivolol at a dose level of 0.25 mg/kg induced a slight reduction in blood pressure (basal value 115±4 and 111±3 mm Hg, respectively) that started slowly and was maintained for 1 h after treatment (Fig. 5A). The two enantiomers elicited a comparable hypotensive effect that was significantly lower as compared to that induced by the racemate at 0.5 mg/kg (Fig. 5A). These data indicate that both D- and L-nebivolol contribute to the centrally mediated reduction in blood pressure induced by nebivolol. Regarding heart rate, i.c.v. D-nebivolol induced a significant bradycardia (basal value 383±12 beats/min) that was comparable, both for entity and duration, to that induced by the racemate at 0.5 mg/kg (Fig. 5B). On the other hand, L-nebivolol induced a significant reduction in heart rate (basal value 366±12 beats/min) but of minor degree and duration compared to other enantiomer (Fig. 5B).

3.3. Effects of L-NAME and atropine treatment on haemodynamic changes induced by intravenous or intracerebroventricular treatment with D/L-nebivolol

The NO synthase inhibitor L-NAME, administered at 5 mg/kg as bolus injection, followed by an i.v. infusion at 0.1 mg/kg/min, induced a significant increase in arterial blood

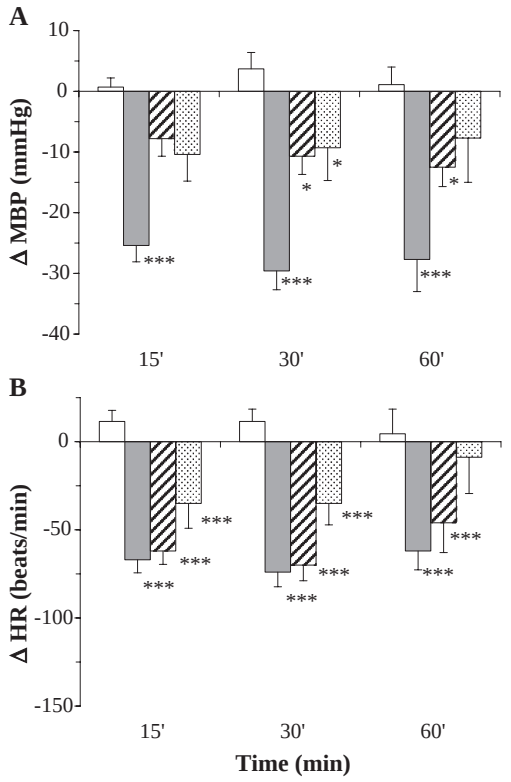

Fig. 5. Effects of i.c.v. D/L-, D- and L-nebivolol on mean blood pressure (A) and on heart rate (B). Effects of vehicle (open bar), D/L-nebivolol at 0.5 mg/kg (grey bar), D-nebivolol (hunched bar) or L-nebivolol (dotted bar) both at 0.25 mg/kg. Data reported are mean differences vs. basal value of 5–6 rats. ***P<0.001 vs. control group.

pressure (about 50 mm Hg; basal value 114±4 mm Hg) starting immediately after the bolus and reaching a maximum 20–25 min later and then remaining constant for at least 60 min. A significant reduction in heart rate (about 120 beats/min; basal value 377±12 beats/min) was also observed in these animals treated with L-NAME.

When administered by i.c.v. route, L-NAME (2.5 mg/kg) induced a significant increase in blood pressure (about 25 mm Hg; basal value 114±4 mm Hg) that was maintained up to 1 h after dosing. I.c.v. L-NAME induced a transient (10–15 min duration) and slight increase of heart rate followed by a significant and long lasting reduction (about 60 beats/min; basal value 342±10 beats/min).

The hypotensive effects elicited by both 1 mg/kg i.v. (Fig. 6A) or 0.5 mg/kg i.c.v. (Fig. 6B) of D/L-nebivolol were completely abolished by the pre-treatment with L-NAME administered by i.v. route. Likewise, blood pressure reducing effects of 0.5 mg/kg D/L-nebivolol i.c.v. were inhibited by the pre-treatment with i.c.v. L-NAME (Fig. 6C).

In these experimental conditions, the effects of D/L-nebivolol on heart rate had not been evaluated since L-NAME alone induced a marked bradycardia.

Atropine (2 mg/kg i.v.) did not modify blood pressure (basal value 115±3 mm Hg) or heart rate (basal value 388±10 beats/min) alone and did not affect the reduction in blood pressure and heart rate induced by i.c.v. administration of 0.5 mg/kg D/L-nebivolol (Fig. 7A/B).

3.4. Effects of L-NAME treatment on haemodynamic changes induced by CGRP and isoprenaline

In order to assess the selectivity of L-NAME-induced blockade of D/L-nebivolol hypotensive effects, its action on isoprenaline or CGRP cardiovascular activities was evaluated. I.v. administration of 300 ng/kg of isoprenaline induced a significant increase in heart rate and a reduction in arterial blood pressure (Table 1), due to an aspecific β-adrenoceptors stimulation (Emorine et al., 1994). In the presence of L-NAME (5 mg/kg+0.1 mg/kg/min) the

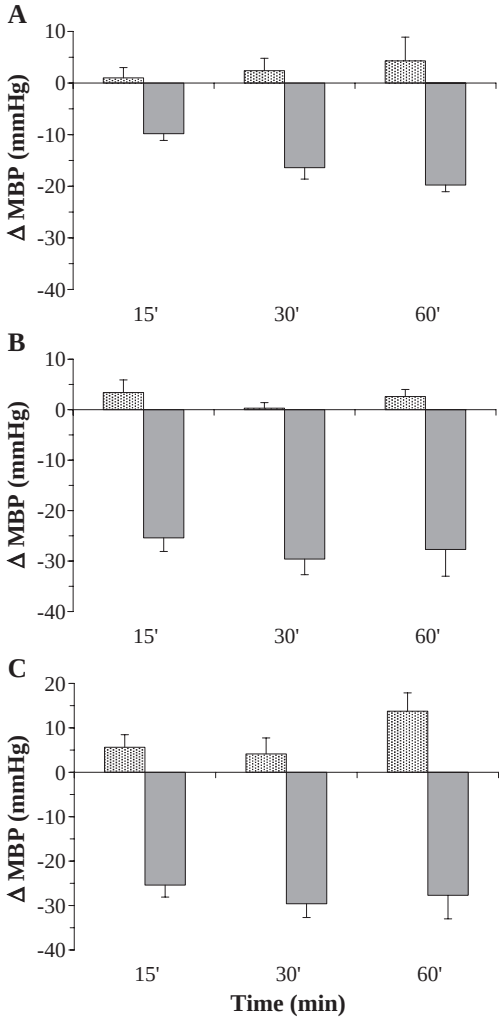

Fig. 6. Effects of i.v. injection of L-NAME (5 mg/kg bolus+0.1 mg/kg/min infusion) on mean blood pressure reduction induced by i.v. injection of 1 mg/kg of D/L-nebivolol (A), or by i.c.v. injection of 0.5 mg/kg of D/L-nebivolol (B). Effects of i.c.v. injection of L-NAME (2.5 mg/kg) on mean blood pressure reduction induced by i.c.v. injection of 0.5 mg/kg of D/L-nebivolol (C). Grey bar: D/L-nebivolol alone, dotted bar: D/L-nebivolol+L-NAME. Data reported are mean differences vs. basal value of 7–9 rats.

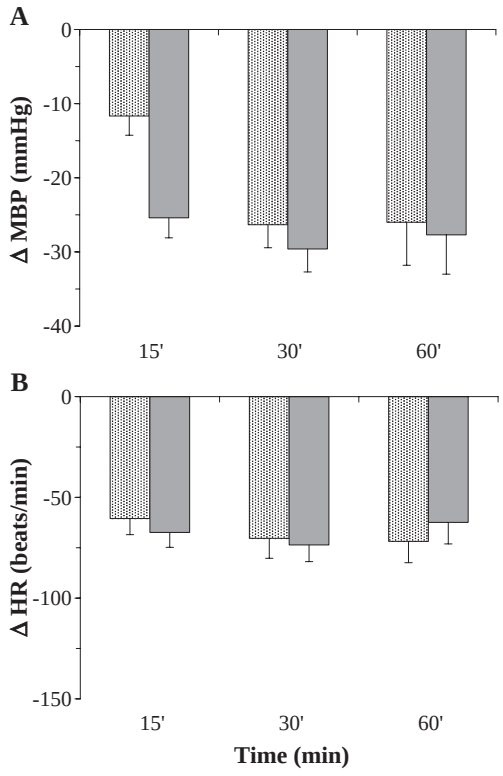

Fig. 7. Effects of i.v. injection of atropine (2 mg/kg) on mean blood pressure (A) and heart rate (B) reduction induced by i.c.v. injection of 0.5 mg/kg of D/L-nebivolol. Grey bar: D/L-nebivolol alone, dotted bar: D/L-nebivolol+ atropine. Data reported are mean differences vs. basal value of 6–9 rats.

haemodynamics effects of isoprenaline were magnified (Table 1).

Furthermore, i.v. administration of 400 ng/kg of CGRP induced a significant reduction in arterial blood pressure and an increase in heart rate (Table 1), due to stimulation of specific receptors (Aiyar et al., 2001). Also the hypotensive effect induced by CGRP was enhanced in the presence of L-NAME treatment as compared to that obtained with CGRP alone (Table 1).

4. Discussion

The results of the present study confirm that D/L-nebivolol has a peculiar cardiovascular profile compared to other β-adrenoceptor antagonists, as it can reduce the arterial blood pressure and heart rate after peripheral and central administration. In terms of hypotensive and bradycardic efficacy, these findings are similar to those reported by Midol-Monnet et al. (1991), who showed that i.c.v. administration of D/L-nebivolol decreased the peripheral vascular resistance whereas metoprolol increased it. Similar hypotensive effects were recorded at a dose 4 times lower by i.c.v. as compared to i.v. route (0.25 mg/kg i.c.v. corresponds to 1 mg/kg i.v., see Figs. 1 and 4), indicating that D/L-nebivolol could modulate arterial blood pressure also through a central pathway. Due to its lipophily, D/L-nebivolol is able to cross the blood–brain barrier and then could contribute to regulate arterial blood pressure in hypertensive patients either by the reduction of peripheral resistance or by the decrease of centrally mediated adrenergic discharge (Cockroft, 2004).

This study also shows that both enantiomers of the drug can produce bradycardic and hypotensive effects when administered by i.c.v. route. The haemodynamic effects of D- and L-enantiomers were different when we used a different way of administration. By a peripheral i.v. injection, haemodynamic effects elicited by D/L-nebivolol were mainly due to D-nebivolol, as indicated by the almost complete inhibition of adrenergic stimulation with isoprenaline elicited by this enantiomer. In addition, only D-nebivolol produced a hypotensive effect comparable to that of racemate. L-Nebivolol did not exert any effect against β-adrenergic stimulation confirming that the β_1-selectivity of the drug can be due to its D-enantiomer. Conversely, when administered by i.c.v. route, both D- and L-nebivolol appeared to be involved in the expression of haemodynamic effects of D/L-nebivolol, particularly for the effects on arterial blood pressure.

Peripheral i.v. treatment with L-NAME, the known inhibitor of L-arginine/NO pathways, resulted in a complete ablation of effects elicited by D/L-nebivolol on arterial blood pressure. The interaction of L-NAME with D/L-nebivolol was completely different from the two other vasoactive agents, isoprenaline and CGRP, confirming its selective mechanism of action. Interestingly, the effects of D/L-nebivolol were abolished by treatment with L-NAME either when D/L-nebivolol was administered by central route or when administered by peripheral i.v. injection. Further, i.c.v. pre-treatment with L-NAME also completely inhibits the effects of D/L-nebivolol on blood pressure. These data suggest that a fully efficient mechanism of NO synthase is necessary for D/L-nebivolol to exert its haemodynamic effects. The involvement of NO in the haemodynamic profile of D/L-nebivolol has been described by several authors (Gao et al., 1991; Cockroft et al., 1995; Bowman et

Table 1
Effects of pre-treatment with L-NAME on CGRP or isoprenaline-induced mean blood pressure reduction and heart rate increase

	Pre-L-NAME		Post-L-NAME[a]	
	Mean blood pressure (mmHg)	Heart rate (beats/min)	Mean blood pressure (mmHg)	Heart rate (beats/min)
CGRP (400 ng/kg)	−23.3±2	37.6±5	−61±11*	54.9±17
Isoprenaline (300 ng/kg)	−40±3	42±4	−64.3±5**	83.6±11*

Mean differences vs. basal/pre-treatment values of 8 rats are reported.
[a] See details in Materials and methods.
* $P<0.01$ vs. pre-L-NAME value.
** $P<0.001$vs. pre-L-NAME value.

al., 1994; Kakoki et al., 1999; Tzemos et al., 2001; Ignarro, 2004). However, our data indicate for the first time an involvement of a central pathway, which requires a working NO synthase in the mechanism of action of D/L-nebivolol. The role of NO as regulator of arterial blood pressure acting through central pathways has been reported (Shapoval et al., 1991; Toda et al., 1993; Horn et al., 1994; Sakai et al., 2000; Lin et al., 1999). In these studies, NO is considered a permanent central regulator of sympathetic nervous system. In fact, NO is continuously produced and when the rate of NO synthesis is reduced, the activity of sympathoexcitatory neurons increases inducing a rise in arterial blood pressure (Harada et al., 1993; Horn et al., 1994). I.c.v. administered D/L-nebivolol could increase the release of NO thus reducing the activity of the sympathetic nervous system and then decreasing the arterial blood pressure and heart rate. The i.c.v. hypotensive effects elicited by L-nebivolol, the enantiomer mainly responsible for the interaction of the drug and the NO system (Gao et al., 1991; Cominacini et al., 2003), support this hypothesis. Furthermore, the para-sympathetic activation in the haemodynamic effects of centrally administered D/L-nebivolol was ruled out by the lack of interference of atropine in these effects.

In conclusion, our data suggest that in the normotensive rat, D/L-nebivolol could affect the cardiovascular system acting both at the central and the peripheral level. Apart from the β_1-adrenoceptor blocking action (mainly due to its D-enantiomer), D/L-nebivolol (as D- and L-enantiomer) affects blood pressure through an action, at the central and the peripheral level, mediated by NO. The NO-mediated central action could work by determining a decrease in the sympathetic discharge.

These characteristics of D/L-nebivolol (blockade of β_1-adrenoceptor, reduction of vascular resistances, decrease in central sympathetic discharge) along with its lack of intrinsic sympathomimetic activity (Janssens et al., 1989; Brixius et al., 2001) could suggest that it is an ideal drug for the treatment of cardiovascular diseases, such as chronic heart failure characterized by an enhanced adrenergic drive (Bristow, 2000).

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References

Aiyar, N., Daines, A.R., Disa, J., Chambers, P.A., Sauermelch, C.F., Quiniou, M.-J., Khandoudi, N., Gout, B., Douglas, S.A., Willette, R.N., 2001. Pharmacology of SB-273779, a nonpeptide calcitonin gene-related peptide 1 receptor antagonist. J. Pharmacol. Exp. Ther. 296, 768–775.

Altwegg, L.A., d'Uscio, L.V., Barandier, C., Cosentino, F., Yang, Z., Lüscher, T.F., 2000. Nebivolol induces NO-mediated relaxations of rat small resistance but not of large elastic arteries. J. Cardiovasc. Pharmacol. 36, 316–320.

Bowman, A.J., Chen, C.P., Ford, G.A., 1994. Nitric oxide mediated venodilator effect of nebivolol. Br. J. Clin. Pharmacol. 38, 199–204.

Bristow, M.R., 2000. β-Adrenergic receptor blockade in chronic heart failure. Circulation 101, 558–569.

Brixius, K., Bundkirchen, A., Bolck, B., Mehlhorn, U., Schwinger, R.H.G., 2001. Nebivolol, bucindolol, metoprolol and carvedilol are devoid of intrinsic sympathomimetic activity in human myocardium. Br. J. Pharmacol. 133, 1330–1338.

Bundkirchen, A., Brixius, K., Bolck, B., Nguyen, Q., Schwinger, R.H.G., 2003. β-Adrenoceptor selectivity of nebivolol and bisoprolol. A comparison of [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol binding sites. Eur. J. Pharmacol. 460, 19–26.

Cockroft, J.R., 2004. Nebivolol: a review. Expert Opin. Pharmacother. 5, 893–899.

Cockroft, J.R., Chowienczyk, P.J., Brett, S.E., Chen, C.PL-H., Dupont, A.G., Van Nueten, L., Wooding, S.J., Ritter, J.M., 1995. Nebivolol vasodilates human forearm vasculature: evidence for an L-arginine/NO-dependent mechanism. J. Pharmacol. Exp. Ther. 274, 1067–1071.

Cominacini, L., Fratta Pasini, A., Garbin, U., Nava, C., Davoli, A., Criscuoli, M., Crea, A., Sawamura, T., Lo Cascio, V., 2003. Nebivolol and its 4-keto derivative increase nitric oxide in endothelial cells by reducing its oxidative inactivation. J. Am. Coll. Cardiol. 42, 1838–1844.

Emorine, L., Blin, N., Strosberg, A.D., 1994. The human β3-adrenoceptor: the search for a physiological function. Trends Pharmacol. Sci. 15, 3–7.

Falciani, M., Rinaldi, B., D'Agostino, B., Mazzeo, F., Rossi, S., Nobili, B., Rossi, F., Filippelli, A., 2001. Effects of nebivolol on human platelet aggregation. J. Cardiovasc. Pharmacol. 38, 922–929.

Gao, Y.S., Nagao, T., Bond, R.A., Janssens, W.J., Vanhoutte, P.M., 1991. Nebivolol induces endothelium-dependent relaxations of canine coronary arteries. J. Cardiovasc. Pharmacol. 17, 964–969.

Gryglewski, R.J., Uracz, W., Marcinkiewicz, E., Jakubowski, A., Malinski, T., 2001. Role of endothelial nitric oxide in pleiotropic action of cardiovascular drugs: nebivolol. In: Gryglewski, R.J., Minuz, P. (Eds.), Nitric Oxide: Basic Research and Clinical Applications. IOS Press, Amsterdam, pp. 57–69.

Harada, S., Tokunaga, S., Momohara, M., Masaki, H., Tagawa, T., Imaizumi, T., Takeshita, A., 1993. Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. Circ. Res. 72, 511–516.

Horn, T., Smith, P.M., McLaughlin, B.E., Bauce, L., Marks, G.S., Pittman, Q.J., Ferguson, A.V., 1994. Nitric oxide action in paraventricular nucleus: cardiovascular and neurochemical implications. Am. J. Physiol. 266, R306–R313.

Ignarro, L.J., 2004. Experimental evidences of nitric oxide-dependent vasodilatory activity of nebivolol, a third generation β-blocker. Blood Press. 13 (Suppl. 1), 3–17.

Janssens, W.J., Van De Water, A., Xhonneux, R., Reneman, R.S., Van Nueten, J.M., Janssen, P.A., 1989. Nebivolol is devoid of sympathomimetic activity. Eur. J. Pharmacol. 159, 89–95.

Kakoki, M., Hirata, Y., Hayakawa, H., Nishimatsu, H., Suzuki, Y., Nagata, D., Suzuki, E., Kikuchi, K., Nagano, T., Omata, M., 1999. Effects of vasodilatory β-adrenoceptor antagonist on eodothelium-derived nitric oxide release in rat kidney. Hypertension 33 (part II), 467–471.

Lin, M.T., Pan, S.P., Lin, J.H., Yang, Y.L., 1999. Central control of blood pressure by nitrergic mechanisms in organum vasculosum laminae terminalis of rat brain. Br. J. Pharmacol. 127, 1511–1517.

Midol-Monnet, M., Davy, M., Heimburger, M., Beslot, F., Cohen, Y., 1991. Cardiovascular effects of intracerebroventricular injections of (±)-nebivolol and its enantiomers—a comparison with those of metoprolol in the rat. J. Pharm. Pharmacol. 43, 504–509.

Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J., 1986. A Stereotaxic Atlas of the Rat Brain, 2nd edition. Plenum Press, New York, pp. 34–36.

Sakai, K., Hirooka, Y., Matsuo, I., Eshima, K., Shigematsu, H., Shimokawa, H., Takeshita, A., 2000. Overexpression of eNOS in

NTS causes hypotension and bradycardia in vivo. Hypertension 36, 1023–1028.

Shapoval, L.N., Sagach, V.F., Pobegailo, L.S., 1991. Nitric oxide influences ventrolateral medullary mechanisms of vasomotor control in the cat. Neurosci. Lett. 132, 47–50.

Toda, N., Kitamura, Y., Okamura, T., 1993. Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs. Hypertension 21, 3–8.

Tzemos, N., Lim, P.O., McDonald, T.M., 2001. Nebivolol reverses endothelial dysfunction in essential hypertension: a randomised, double-blind, cross-over study. Circulation 104, 511–514.

Van de Water, A., Xhonneux, R., Reneman, R.S., Janssen, P.A.J., 1988. Cardiovascular effects of dl-nebivolol and its enantiomers—a comparison with those of atenolol. Eur. J. Pharmacol. 156, 95–103.