Comparison of the effects of nadolol and bisoprolol on the isoprenaline-evoked dilatation of the dorsal hand vein in man

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Aims We attempted to explore the possible differential involvement of β-adrenoceptor subtypes in the dilator response of the human dorsal hand vein to isoprenaline by examining the ability of bisoprolol, a selective β_1-adrenoceptor antagonist, and nadolol, a nonselective β_1/β_2-adrenoceptor antagonist, to antagonize the response.

Methods Twelve healthy male volunteers participated in four weekly sessions. In the preliminary session a dose-response curve to the vasoconstrictor effect of phenylephrine was constructed and the dose producing 50–75% maximal response was determined for each individual. In each of the remaining three (treatment) sessions, nadolol (40 mg), bisoprolol (5 mg) or placebo was ingested, and isoprenaline hydrochloride (3.33–1000 ng min^{-1}) was infused locally into the dorsal hand vein along with a constant dose of phenylephrine hydrochloride (to preconstrict the vein) 2 h after the ingestion of the drugs. Changes in vein diameter were monitored with the dorsal hand vein compliance technique. Subjects were allocated to treatment session according to a double-blind balanced cross-over design. Systolic and diastolic blood pressure, and heart rate were also measured.

Results Isoprenaline produced dose-dependent venodilatation which was antagonized by nadolol but remained unaffected by bisoprolol (ANOVA with repeated measures: $P<0.025$; Dunnett's test: placebo *vs* nadolol, $P<0.01$; placebo *vs* bisoprolol, $P=$NS). Mean log ED_{50} (ng min^{-1}) was significantly increased in the presence of nadolol and remained unchanged in the presence of bisoprolol (ANOVA, $P<0.025$; Dunnett's test: placebo *vs* nadolol, $P<0.005$; placebo *vs* bisoprolol, $P=$NS; differences between mean log ED_{50} [95% CI]: placebo *vs* bisoprolol −0.11 [-0.38, 0.16], placebo *vs* nadolol 0.32[0.09, 0.72], bisoprolol *vs* nadolol −0.43 [-0.71, −0.15]). Mean E_{max} did not differ in the three treatment conditions.

Conclusions The failure of bisoprolol to attenuate isoprenaline-evoked venodilatation in the human dorsal hand vein argues against the involvement of a β_1-adrenoceptor-mediated component in the isoprenaline-evoked venodilatory responses. The possibility cannot be excluded that the consequences of β_1-adrenoceptor blockade by bisoprolol might have been obscured by a possible venodilator effect of bisoprolol.

Keywords: β-adrenoceptors, bisoprolol, dorsal hand vein, nadolol, noradrenaline

Introduction

The human dorsal hand vein contains both β- and α-adrenoceptors [1-3] and there is evidence that both α_1- and α_2-adrenoceptors mediate constrictor responses [3, 4] and β_2-adrenoceptors mediate dilator responses [3, 5]. It is more controversial, however, whether there are also venodilator β_1-adrenoceptors in the dorsal hand vein. Attempts to answer this question have been impeded by the lack of availability of selective β_1-adrenoceptor agonists suitable for administration to human subjects, and researchers have been forced to use a more indirect approach involving selective β_1-adrenoceptor antagonists

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and nonselective adrenoceptor agonists. Two approaches have been adopted: (a) investigation of the effect of selective β_1-adrenoceptor antagonists on venodilator responses to the nonselective β_1-/β_2-adrenoceptor agonist isoprenaline, and (b) investigation of the effect of selective β_1-adrenoceptor antagonists on venoconstrictor responses to the nonselective α/β-adrenoceptor agonist noradrenaline.

In early studies, practolol, a β_1-adrenoceptor antagonist with some intrinsic sympathomimetic (partial agonist) activity, was used. It was reported that practolol could both antagonize isoprenaline-evoked venodilatation and potentiate noradrenaline-evoked venoconstriction [1], suggesting the involvement of venodilator β_1-adrenoceptors in the responses to both isoprenaline and noradrenaline. More recently, we have reinvestigated the question of the probable involvement of masked β_1-adrenoceptors in the venoconstrictor response to noradrenaline by using bisoprolol, a highly selective β_1-adrenoceptor antagonist lacking in intrinsic sympathomimetic activity [6–8]. We found that bisoprolol failed to potentiate the constrictor response to noradrenaline, and that it antagonized the constrictor responses both to noradrenaline and the selective α_1-adrenoceptor agonist phenylephrine [9, 10]. These results suggest that bisoprolol, similarly to another selective β_1-adrenoceptor antagonist, nebivolol, may dilate the vein. The venodilatation evoked by nebivolol is mediated via the release of nitric oxide [11, 12].

In the present experiment, we extended our previous work with bisoprolol by examining its effect on isoprenaline-evoked venodilatation. Nadolol, a nonselective β_1/β_2-adrenoceptor antagonist, was used as a control. Some of these results have been communicated to the British Pharmacological Society [13].

Methods

Ethical considerations

The study protocol was approved by the University of Nottingham Medical School Ethics Committee. All volunteers gave their written informed consent following a verbal explanation of the study and after reading a detailed information sheet.

Subjects

Twelve healthy male volunteers aged 18–30 years (mean $\pm$s.e mean, 21.3 ± 2.7) and weighing 58–86 kg (mean $\pm$s.e mean, 74.8 ± 3.2) participated. Each subject completed a brief medical history and underwent a complete physical examination. Subjects had not participated in drug studies within 3 months of the start of the study, and had not used any drug within the 14 days preceding the study. They were requested to stop smoking and to avoid drinking alcohol, coffee and other caffeine-containing beverages for at least 12 h before each experimental session. All subjects were advised to have a light breakfast 2 h before the experimental sessions.

Drugs

Isoprenaline hydrochloride (Saventrine IV^R) was obtained from Pharmax Ltd, Kent, UK, phenylephrine hydrochloride (Phenylephrine Injection^R) from Boots Pharmaceuticals, Nottingham, UK, bisoprolol fumarate (Monocor*5^R) from Lic. E. Merck, Darmstadt, Germany, nadolol (Corgard^R) from Squibb. The sterile solutions of isoprenaline hydrochloride and phenylephrine hydrochloride were infused locally into the vein at a constant rate of 0.3 ml min^{-1} and over the following dose range: isoprenaline hydrochloride 3.33–1000 ng min^{-1}, phenylephrine hydrochloride 0.033–10 μg min^{-1}. Bisoprolol 5 mg, nadolol 40 mg, and lactose placebo were prepared in identical capsules for double-blind administration.

Tests

The dorsal hand vein compliance technique The dorsal hand vein compliance technique, as modified by Aellig [14], was used as described previously [15]. Each period of drug-infusion consisted of an initial 3 min with the cuff deflated, followed by a further 2–4 min with the cuff inflated (i.e. a sufficient period of time to ensure that the signal from the Linear Variable Differential Transformer (LVDT) had reached plateau). The baseline venodilatation during saline infusion with the cuff inflated was defined as 100% relaxation, the recording with the cuff not inflated was defined as 100% constriction. As the vein has no resting tone, it was necessary to preconstrict it with phenylephrine prior to the infusion of isoprenaline in order to be able to record dilator responses [16]. Venodilator responses to isoprenaline following the preconstriction with phenylephrine were defined as a percentage of the baseline obtained during initial saline infusion by the formula $[(Z-Y)/(X-Y)] \times 100$, where Z is vein diameter during the coinfusion of each dose of isoprenaline and a fixed ('preconstricting'') dose of phenylephrine, X is vein diameter ('baseline') during infusion of saline and Y is vein diameter (preconstricted) during infusion of phenylephrine. Drug solutions were infused at a constant rate (0.3 ml min^{-1}).

Cardiovascular measures Systolic and diastolic blood pressure were measured using a mercury sphygmomanometer, and heart rate by feeling the pulse of the radial artery at the wrist for 1 min. All measurements were taken on the arm opposite to the one used for the pharmacological testing.

Experimental design

Subjects were allocated to systemic treatments and sessions according to a double-blind balanced design. Each volunteer participated in four experimental sessions at weekly intervals. In the first (preliminary) session a dose–response curve to phenylephrine was constructed and the dose producing 50–75% maximal response was determined for each individual subject; this dose was used in subsequent experimental sessions in order to 'set the baseline' for measuring the responses to isoprenaline. Each of the remaining three sessions was associated with one of the following treatments: bisoprolol (5 mg), nadolol (40 mg), or placebo. After a 30 min acclimatization period, the first cardiovascular testing (blood pressure and heart rate; measurement I) was carried out. Then the subject ingested the drug capsule. 2 h later the second cardiovascular testing (II) was carried out, followed by a 30 min period of saline infusion during which baseline venous diameter was recorded. This was followed by the third cardiovascular testing (III). Then the 'baseline-setting' dose of phenylephrine was infused for 5 min, followed by the coinfusion of phenylephrine + isoprenaline (6 doses) for 30 min. Finally, the fourth cardiovascular testing (IV) was carried out. The timings of start of local infusion and post-treatment tests were based on the single-dose pharmacokinetics of bisoprolol and nadolol; it has been reported that peak plasma concentration is attained 2–4 h after oral administration of a single dose of bisoprolol [17, 18], and nadolol [19, 20, 21].

Data analysis

Dorsal hand vein responses The raw data were analysed with two-way analysis of variance (ANOVA, dose of agonist; systemic drug treatment) with repeated measures on both factors. When a significant overall main effect of drug treatment was identified, individual comparisons were made between placebo *vs* bisoprolol and placebo *vs* nadolol with Dunnett's test. The individual dose–response curves obtained in each subject were also analysed by fitting a rectangular hyperbolic function to the data using a computer program based on Wilkinson's method [22]. This analysis yields estimates of the maximal response (E_{max}) and the dose producing the half-maximal response (ED_{50}). The analysis also provides the index of determination (p^2) for each curve; p^2 expresses the proportion of the data variance accounted for by the fitted function. The distribution of the ED_{50} values was normalized by logarithmic transformation, and the geometric mean was calculated for each of the three dose–response curves. Dunnett's test was used to compare the effects of bisoprolol *vs* placebo, and nadolol *vs* placebo on E_{max} and log ED_{50}. The degree of antagonism of the responses by bisoprolol and nadolol was expressed in two ways: by calculating the percentage change in geometric mean ED_{50} in the presence of bisoprolol and nadolol, and by calculating the dose-ratio [15]. 95% confidence intervals were calculated for the differences between the dose–response curve parameters seen under the three treatment conditions.

Cardiovascular measures The effects of the three systemic treatments on the cardiovascular measures were calculated both in the absence (difference between measurements II and I) and in the presence (difference between measurements IV and III) of the infusion of isoprenaline (Table 4). One-way ANOVA (repeated measures) was used to detect any significant effect of systemic drug treatment; in the case of significant treatment effects, Dunnett's test was used to compare the effects of the active treatments with those of placebo. 95% confidence intervals were calculated for the differences between the cardiovascular measures seen under the three treatment conditions, both in the presence and in the absence of isoprenaline.

A probability level of $P<0.05$ was considered as being of significance for all statistical tests.

Results

Dorsal hand vein responses

Venous diameter, recorded following the administration of systemic treatments, both prior to and following the local infusion of saline/drug solutions, is shown in Table 1. The effects of the systemic drug treatments on the dose–response curves to isoprenaline are shown in

Table 1 Venous diameter (mm, mean ± s.e. mean) recorded following the administration of systemic treatments and during the infusion of saline ('baseline'), phenylephrine hydrochloride solution ('preconstriction'), and phenylephrine hydrochloride + six different doses of isoprenaline hydrochloride (1–6). See text for details.

			Phenylephrine + isoprenaline					
	Saline	*Phenylephrine*	*1*	*2*	*3*	*4*	*5*	*6*
Placebo	0.73 ± 0.11	0.19 ± 0.05	0.22 ± 0.05	0.26 ± 0.05	0.34 ± 0.06	0.44 ± 0.07	0.52 ± 0.08	0.59 ± 0.09
Bisoprolol 5 mg	0.76 ± 0.07	0.24 ± 0.05	0.28 ± 0.06	0.32 ± 0.05	0.36 ± 0.05	0.43 ± 0.07	0.52 ± 0.07	0.62 ± 0.07
Nadolol 40 mg	0.78 ± 0.11	0.14 ± 0.03	0.14 ± 0.03	0.17 ± 0.04	0.23 ± 0.04	0.31 ± 0.07	0.38 ± 0.07	0.47 ± 0.09

Figure 1. Isoprenaline evoked dose-dependent venodilator responses, which were antagonized by nadolol and remained unaffected by bisoprolol. ANOVA showed significant main effects of both dose of isoprenaline ($P<0.0001$) and of the systemic drug treatments ($P<0.025$), and a significant interaction ($P<0.05$). Dunnett's test revealed a significant effect of nadolol, but not of bisoprolol: placebo *vs* bisoprolol ($P=$NS); placebo *vs* nadolol ($P<0.01$). In the case of the individually fitted curves ($n=12$) the value of p^2 ranged from 0.92 to 0.99 (median 0.97) in the presence of placebo, from 0.72 to 0.99 (median 0.91) in the presence of bisoprolol, and from 0.88 to 0.99 (median 0.95) in the presence of nadolol. The estimated parameters of the dose–response curves ($n=12$) are shown in Table 2. Mean log ED_{50} was significantly increased in the presence of nadolol and remained unchanged in the presence of bisoprolol (ANOVA, $P<0.025$; Dunnett's test: placebo *vs* nadolol: $P<0.005$; placebo *vs* bisoprolol, $P=$NS).The effects of the two active treatments on the log ED_{50}, compared using Bonferroni-corrected *t*-test, differed significantly from one another ($P<0.05$). Mean E_{max} did not differ in the three treatment conditions (ANOVA, $P=$NS). The geometric mean ED_{50} was increased by approximately 207% in the presence of nadolol, and decreased by approximately 22% in the presence of bisoprolol, and the dose ratios were: placebo *vs* nadolol 2.08, and placebo *vs* bisoprolol 0.78. Table 3 shows the differences (mean, 95% CI, $n=12$) between the effect of placebo and active drug treatments, and between the two active drug treatments, on the parameters of the dose–response curves.

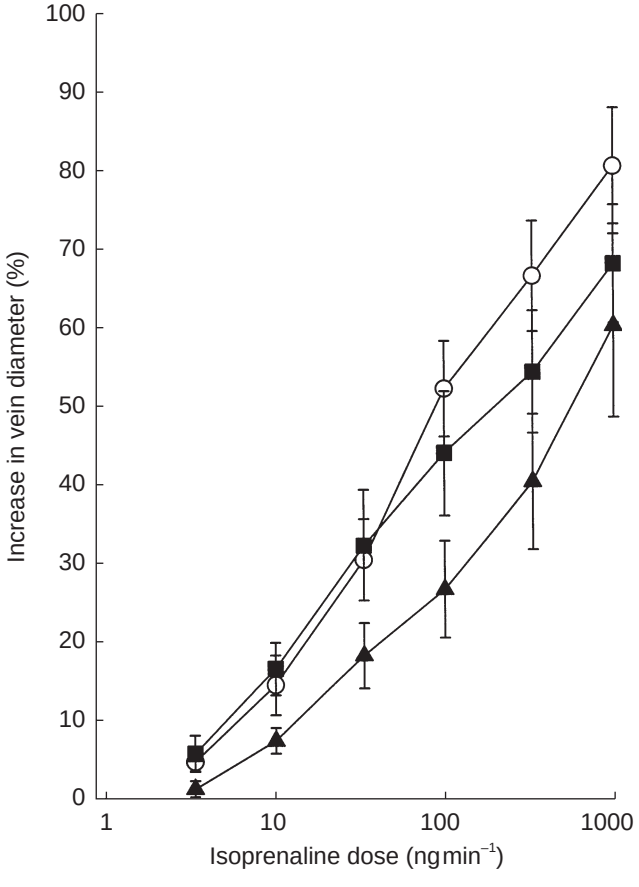

Figure 1 Dose–response curves for the venodilator effect of isoprenaline during local infusion into the superficial dorsal hand vein (cuff pressure 45 mmHg) 2 h after ingestion of placebo (○), bisoprolol 5 mg (■), and nadolol 40 mg (▲); mean ± s.e. mean, $n=12$. 100% response was defined as the recording during saline infusion with the cuff inflated.

Cardiovascular measures

The effects of bisoprolol, nadolol, and placebo on cardiovascular measures are summarized in Table 4. Both bisoprolol and nadolol decreased heart rate (ANOVA, $P<0.0025$; Dunnett's test: placebo *vs* bisoprolol $P<0.005$; placebo *vs* nadolol, $P<0.01$), and systolic blood pressure (ANOVA, $P<0.0025$; Dunnett's test: placebo *vs* bisoprolol, $P<0.005$; placebo *vs* nadolol, $P<0.01$). There was no significant effect of bisoprolol or nadolol on diastolic blood pressure (ANOVA, $P=$NS). There was no significant difference between the effects of nadolol and bisoprolol on any of the cardiovascular measures. The mean differences (95% CI) between the effect of placebo and each active drug treatment and between the two active treatments on the cardiovascular measures are shown in Table 5.

The effects of isoprenaline on cardiovascular measures in the presence of placebo, bisoprolol, and nadolol are summarized in Table 4. In the presence of placebo, isoprenaline increased heart rate and systolic blood

Table 2 Parameters of the dose-response curves.

	Log ED$_{50}$ *(mean ± s.e. mean)*	*ED$_{50}$* *(geometric mean)*	*E_{max} (%)* *(mean ± s.e. mean)*
Placebo	1.79 ± 0.10	60.98	84.13 ± 8.09
Bisoprolol 5 mg	1.68 ± 0.11	47.45	68.07 ± 7.91
Nadolol 40 mg	2.1 ± 0.12★	126.62	68.09 ± 12.81

ED_{50}: ng min^{-1}
★$P<0.005$ (difference from placebo condition).

Table 3 Parameters of dose–response curves: differences (mean, 95% CI) between placebo (Pl) and bisoprolol 5 mg (BIS5), placebo and nadolol 40 mg (NAD40), and bisoprolol 5 mg and nadolol 40 mg.

	Log ED$_{50}$ (ng min^{-1})	*E_{max} (%)*
Pl/BIS5	−0.11 (−0.38, 0.16)	−16.06 (−39.11, 6.99)
Pl/NAD40	0.32 (0.09, 0.72)	−16.05 (−37.70, 5.62)
BIS5/NAD40	−0.43 (−0.71, −0.15)	−0.02 (−27.36, 27.32)

pressure, and reduced diastolic blood pressure. These effects were significantly attenuated by both bisoprolol and nadolol. Table 5 shows the differences (mean 95% CI, $n=12$) between placebo and active drug treatments on the cardiovascular measures.

Discussion

The doses of the two β-adrenoceptor antagonists used in the study were selected on the basis of published reports on the effectiveness of these drugs in man. Bisoprolol 5 mg and nadolol 40 mg have been shown to produce a similar degree of antagonism (16.8% and 22.9%, respectively) of exercise-induced tachycardia, a β_1-adrenoceptor-mediated response [23], and bisoprolol 5 mg does not block β_2-adrenoceptors in man [24].

An oral dose (40 mg) of nadolol antagonized the venodilator responses to isoprenaline leading to a rightward shift in the dose–response curves of isoprenaline, whereas a single oral dose (5 mg) of bisoprolol was without a significant effect. Both nadolol and bisoprolol reduced heart rate and blood pressure, in agreement with previous reports [25–27] and consistent with the well-documented effects of these drugs on the cardiovascular system, i.e slowing of the heart and decrease in myocardial contractility [28].

It was predicted that bisoprolol, a highly selective β_1-adrenoceptor antagonist [6, 7], would either antagonize or not affect the dilator responses to isoprenaline, depending on the contribution of venodilator β_1-adrenoceptors to the response. Bisoprolol (5 mg) had no effect on isoprenaline-evoked venodilatation, consistent with the hypothesis that β_1-adrenoceptors do not contribute significantly to isoprenaline-evoked venodilatation in the dorsal hand vein. It should be noted, however, that bisoprolol may have additional pharmacological properties, which may have masked the antagonism of the responses to isoprenaline. Indeed, in a separate study, we observed that bisoprolol could antagonize both noradrenaline- and phenylephrine-evoked venoconstriction in a dose dependent manner [9]. As there is evidence that the venoconstrictor responses to noradrenaline and phenylephrine are mediated by α_1- and α_2-adrenoceptors [3], and bisoprolol has no affinity for these receptors [6, 7], the antagonism of the constrictor responses by bisoprolol is likely to reflect functional rather than competitive antagonism due to a possible venodilator effect of the drug [9]. Indeed, there is some indirect evidence that bisoprolol may have exerted such an effect in the present study. There was a tendency for bisoprolol to reduce isoprenaline-induced venodilatation at the higher concentrations of isoprenaline. This observation may reflect an artefactual reduction in the sizes of the responses, due to

Table 4 Cardiovascular parameters prior to (A) and following (B) the infusion of isoprenaline.

	A *Change from pretreatment baseline (mean ± s.e. mean)*	*B* *Change from preinfusion value (mean ± s.e. mean)*
Heart rate (beats min^{-1})[1]		
Placebo	−5.00 ± 1.72	14.33 ± 2.70
Bisoprolol 5 mg	−15.33 ± 2.91**	4.83 ± 1.85*
Nadolol 40 mg	−12.50 ± 1.80*	1.08 ± 1.24*
Systolic BP (mmHg)[2]		
Placebo	0.42 ± 2.34	14.42 ± 3.69
Bisoprolol 5 mg	−14.17 ± 2.94**	1.25 ± 1.52*
Nadolol 40 mg	−9.17 ± 1.35*	0.42 ± 2.08*
Diastolic BP (mmHg)[3]		
Placebo	2.33 ± 1.92	−8.92 ± 2.41
Bisoprolol 5 mg	−2.92 ± 1.41	−3.17 ± 0.88*
Nadolol 40 mg	−1.67 ± 1.68	−1.00 ± 1.04*

[1,2,3]: significance of treatment effect from the ANOVA: 1: $P<0.0001$; 2: $P<0.0001$; 3: $P<0.0025$.
Asterisks: significance of Dunnett's test (difference from placebo condition): *$P<0.01$; **$P<0.005$.

Table 5 Effect of systemic drug treatments in the absence and presence of isoprenaline on the cardiovascular measures: heart rate (HR: beats min^{-1}), systolic blood pressure (SBP: mmHg), and diastolic blood pressure (DBP: mmHg), difference (mean, 95% CI, $n=12$) between placebo (Pl) and bisoprolol 5 mg (BIS5), placebo and nadolol 40 mg (NAD40), and bisoprolol 5 mg and nadolol 40 mg.

	HR (beats min^{-1})	*SBP (mmHg)*	*DBP (mmHg)*
In the absence of isoprenaline			
Pl *vs* BIS5	−10.3 (−16.4, −4.2)	−14.6 (−23.4, −5.8)	−5.3 (−8.8, −1.7)
Pl *vs* NAD40	−7.5 (−11.5, −3.5)	−9.58 (−16.42, −2.75)	−4.0 (−9.0, 1.0)
BIS5 *vs* NAD40	2.8 (−4.0, 9.6)	5.0 (−1.1, 11.1)	1.3 (−2.7, 5.2)
In the presence of isoprenaline			
Pl *vs* BIS5	−9.5 (−13.73, −5.27)	−13.2 (−21.2, −5.1)	5.8 (1.1, 10.4)
Pl *vs* NAD40	−13.3 (−18.5, −8.0)	−14.0 (−21.8, −6.2)	7.9 (2.3, 13.6)
BIS5 *vs* NAD40	−3.8 (−8.1, 0.6)	−0.8 (−6.9, 5.2)	2.2 (−0.1, 4.4)

a 'floor effect' [29], manifesting when the sizes of the responses were enhanced by the added effect of bisoprolol-induced venodilatation. A possible venodilator effect of bisoprolol is not excluded by the lack of effect of the drug on the diameter of the untreated vein, since the dorsal hand vein has no tone at rest [16, 30], and hence it has to be preconstricted to reveal the dilator effect of any drug applied locally or systemically [11, 16, 30].

In conclusion, a single oral dose (40 mg) of nadolol attenuated isoprenaline-evoked venodilatation in the dorsal hand vein consistent with its β-adrenoceptor antagonistic properties, while the highly selective β_1-adrenoceptor antagonist bisoprolol, a drug recommended for the characterization of β_1-adrenoceptors [6], failed to do so. Although the lack of effect of bisoprolol on the responses to isoprenaline would be consistent with the absence of a significant β_1-adrenoceptor-mediated component in the isoprenaline-evoked venodilatation, the possibility cannot be excluded that the consequences of β_1-adrenoceptor blockade by bisoprolol might have been obscured by some other effect of bisoprolol (e.g. venodilatation).

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