Bisoprolol attenuates noradrenaline- and phenylephrine-evoked venoconstriction in man in vivo

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Aims The aim of this study was to examine the effects of bisoprolol (BIS), a selective β1-adrenoceptor antagonist without partial agonistic activity, on noradrenaline- and phenylephrine-evoked venoconstriction in man using the dorsal hand vein compliance technique.

Methods Twelve healthy male volunteers participated in three weekly experimental sessions. Subjects were allocated to treatments and sessions on a double-blind basis. In each session either BIS 5 mg (BIS5), or BIS 10 mg (BIS10), or placebo was administered orally, and noradrenaline acid tartrate (0.1–33.3 ng min⁻¹) followed by phenylephrine hydrochloride (0.033–10 mg min⁻¹) was infused into the dorsal hand vein. Systolic and diastolic blood pressure and heart rate were also measured.

Results Both noradrenaline and phenylephrine produced dose-dependent venoconstriction: the geometric mean ED₅₀ for noradrenaline was 3.21 ng min⁻¹ and for phenylephrine 135.04 ng min⁻¹; the potency ratio (noradrenaline/phenylephrine) was 42. Both BIS5 and BIS10 significantly decreased the venoconstriction to noradrenaline (ANOVA; P < 0.005), and to phenylephrine (ANOVA; P < 0.001). The antagonism of the venoconstrictor responses was also reflected in a significant increase in logED₅₀ values for both noradrenaline (ANOVA; P < 0.005), and phenylephrine (ANOVA; P < 0.0025) in the presence of both doses of BIS. Both doses of BIS significantly decreased heart rate (ANOVA; P < 0.0001), and systolic blood pressure (ANOVA; P < 0.0025).

Conclusions Bisoprolol can antagonize α1-adrenoceptor mediated venoconstriction in the human dorsal hand vein in vivo through a mechanism which remains to be elucidated.

Keywords: bisoprolol, noradrenaline, phenylephrine, dorsal hand vein, β1-adrenoceptor antagonist

Introduction
Like other superficial veins [1–3], the dorsal hand vein in man contains both β- and α-adrenoceptors [4–6]. There is evidence that β2-adrenoceptors mediate vasoconstriction [1, 6], and both α1- and α2-adrenoceptors mediate venoconstriction [6, 7]. The effects of β-adrenoceptor antagonists have been studied on both isoprenaline-evoked vasoconstriction and on noradrenaline- and phenylephrine-evoked venoconstriction. It is well documented that isoprenaline-evoked vasoconstriction is antagonized by β-adrenoceptor antagonists such as propranolol [4, 8, 9], consistent with the interaction of both the agonist and the antagonist with β-adrenoceptors. The effects of β-adrenoceptor antagonists on noradrenaline- and phenylephrine-evoked vasoconstrictor responses are more controversial. Thus it has been reported that the vasoconstrictor response to noradrenaline can be potentiated by both propranolol and practolol [4, 10]. This observation has been interpreted as evidence for the blockade of vasoconstrictor β-adrenoceptors which attenuate the constrictor response to noradrenaline. Indeed the effectiveness of practolol, a selective β2-adrenoceptor antagonist, could be used as an argument in favour of the existence of venodilator β2-adrenoceptors which mediate venoconstriction [10]. Propranolol, a non-selective β-adrenoceptor antagonist, has been reported to be without any effect on phenylephrine-evoked vasoconstriction [11], indicating the lack of involvement of β2-adrenoceptors in the vasoconstrictor response to phenylephrine. On the other hand, nebivolol, another selective β1-adrenoceptor antagonist, has been shown to be effective in antagonizing phenylephrine-evoked vasoconstriction [11, 12]. This latter observation has been shown to be due to the release of nitric oxide from the vascular endothelium by nebivolol [11, 12].

In the present study we examined the effects of two oral doses of bisoprolol (BIS), a highly selective β1-adrenoceptor antagonist [13, 14], on constrictor responses to the α1-adrenoceptor agonists noradrenaline and phenylephrine, using the dorsal hand vein compliance technique [15] in healthy volunteers. Some of these results have been communicated to the British Pharmacological Society [16].
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
The study was conducted in 12 healthy male volunteers aged 18–28 years (mean ± s.d., 21.3 ± 3.2 years), body weight 62–85 kg (mean ± s.d., 71.1 ± 5.9 kg). Each subject completed a brief medical history and underwent a complete physical examination. Subjects had not participated in drug studies within 3 months before the start of the present 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 meal 2 h before the experimental sessions. All subjects indicated compliance with these requests.

Drugs
Noradrenaline acid tartrate (Levophed®) was obtained from Sanofi-Winthrop, Guildford, Surrey, UK, phenylephrine hydrochloride (Phenylephrine Injection®) from Boots Pharmaceuticals, Nottingham, UK, bisoprolol fumarate (Monocor 5®, Monocor 10®) from Lic. E. Merck, Darmstadt, Germany. The sterile solutions of noradrenaline acid tartrate and phenylephrine hydrochloride were administered locally to the veins at a constant rate of 0.3 ml min⁻¹ and over the following dose ranges: noradrenaline acid tartrate 0.1–33 ng min⁻¹; phenylephrine hydrochloride 0.033–10 µg min⁻¹. BIS (5 and 10 mg) and lactose placebo were prepared in identical capsules for double-blind oral administration.

Tests
The dorsal hand vein compliance technique. The dorsal hand vein compliance technique, as modified by Aellig [15], was used as described previously [17]. Each period of drug-infusion consisted of an initial 3 min period with the cuff deflated, followed by a further 2–4 min period with the cuff inflated (i.e. a sufficient period of time to ensure that the signal from the linear variable differential transformer had reached plateau). Increasing concentrations of the agonist were given at a constant infusion rate (0.3 ml min⁻¹). A washout period of 15 min was allowed between the infusions of the highest dose of noradrenaline and the first dose of phenylephrine by switching to a separate infusion pump connected to the system. In each experiment, vein size returned to baseline during the washout period (see Results).

Data analysis
Dorsal hand vein responses. The data obtained with the two locally infused agonists were analyzed separately. The raw data were analyzed with two-way analysis of variance (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 and each dose of BIS with Dunnert’s test. The individual dose-response curves obtained in each subject were also analyzed to estimate the maximal response (Emax) and the dose producing the half-maximal response (ED50), using a computer program based on Wilkinson’s method [29]. This analysis also provided the index of determination (p²) for each curve; p² expresses the proportion of the data variance accounted for by the fitted function [21]. The distribution of the ED50 values was normalized by logarithmic transformation, and the geometric mean was calculated for each of the six dose-response curves.
Noradrenaline dose (ng min⁻¹) parameters of the dose-response curves are shown in ways: by calculating the percentage change in geometric of antagonism of the responses by BIS was expressed in two placebo and in the presence of each dose of BIS. The degree between the values of these parameters in the presence of test was used to derive mean difference (and 95% CI) between the values of these parameters in the presence of BIS and the presence of each dose of BIS. The degree of antagonism of the responses by BIS was expressed in two ways: by calculating the percentage change in geometric mean E₅₀ in the presence of each BIS dose, and by calculating the dose-ratio. The dose-ratio was calculated by taking the anti log of mean change in log E₅₀. A similar procedure was used to calculate the potency ratio of the two agonists (noradrenaline/phenylephrine).

Cardiovascular measures Analysis of variance (repeated measures) and Dunnett’s test were used to compare the effects of the two doses of BIS on cardiovascular measures.

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

Results

Dorsal hand vein responses

Venous diameter was recorded prior to the application of each agonist and compared between the three sessions (Table 1). There was no significant effect of systemic treatments on venous diameter (ANOVA with repeated measures; prior to noradrenaline: F(2,22)=0.20, P>0.1; prior to phenylephrine: F(2,22)=0.18, P>0.1).

Effects of agonists

The dose-response curve to noradrenaline in the presence of placebo is shown in Figure 1, and the dose-response curve to phenylephrine in the presence of placebo is shown in Figure 2. In the case of the individually fitted curves (n=12), the proportion of the data variance accounted for by the fitted function (p²) ranged from 0.83 to 0.99 (median 0.97) in the case of noradrenaline, and from 0.75 to 0.99 (median 0.95) in the case of phenylephrine. The estimated parameters of the dose-response curves are shown in Figure 3: the mean log E₅₀ for noradrenaline was significantly less than that for phenylephrine (Student’s t-test, paired comparison: t=13.28, df=21, P<0.0001). The geometric mean E₅₀ for noradrenaline was 2.38% of the geometric mean E₅₀ for phenylephrine, and the potency ratio (noradrenaline/phenylephrine) was 42.

Table 1 Venous diameter (mm; mean ± s.e. mean, n=12) at a congestion pressure of 45 mmHg 2 h after ingestion of placebo (Pl), bisoprolol 5 mg (BIS5) and bisoprolol 10 mg (BIS10), and prior to the local infusion of the agonists noradrenaline (NA) and phenylephrine (PHE). Right-hand columns show mean differences (95% CI) between each dose of BIS and Pl.

<table>
<thead>
<tr>
<th>Mean ± s.e. mean (mm)</th>
<th>Mean difference (95% CI) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pl vs BIS5</td>
</tr>
<tr>
<td>(a) Prior to NA</td>
<td>0.08 ± 0.16</td>
</tr>
<tr>
<td>(b) Prior to PHE</td>
<td>0.87 ± 0.16</td>
</tr>
</tbody>
</table>

Figure 2. Dose-response curves for the venoconstrictor effect of phenylephrine during local infusion into the superficial dorsal hand vein (cuff pressure 45 mmHg) 2 h after ingestion of placebo (C) and of bisoprolol 5 mg (A) and 10 mg (B): mean ± s.e. mean = 12. 100% response was defined as abolition of the venous oscillation produced by the inflation of the cuff.

Phenylephrine. Figure 2 shows the dose-response curves to phenylephrine in the presence of placebo and BIS5 and BIS10. Both doses of BIS produced significant increases in log E_max and E_max obtained in the presence of each dose of BIS and the corresponding values obtained in the presence of placebo. Both doses of BIS produced significant increases in log E_max compared with placebo (placebo vs BIS5: t = 3.04, df = 22, k = 3, P < 0.01; placebo vs BIS10: t = 4.37, df = 22, k = 3, P < 0.005). The geometric mean E_max (c.f. Figure 3) was increased by 155% and 289% in the presence of BIS and BIS10, respectively, and the dose ratios were 2.55 for placebo/BIS5, and 3.89 for placebo/BIS10.

Cardiovascular measures
The effects of BIS5, BIS10 and placebo on cardiovascular measures are shown in Table 3. Both doses of BIS decreased heart rate (ANOVA with repeated measures: F(2,22) = 21.17, P < 0.0001; Dunnett’s test: placebo vs BIS: t = 3.92, df = 22, k = 3, P < 0.005; placebo vs BIS10: t = 6.46, df = 22, k = 3, P < 0.005), and systolic blood pressure (ANOVA with repeated measures: F(2,22) = 9.63, P < 0.0025; Dunnett’s test: placebo vs BIS: t = 3.27, df = 22, k = 3, P < 0.005; placebo vs BIS10: t = 4.17, df = 22, k = 3, P < 0.005). There was no statistically significant effect of BIS on diastolic blood pressure (ANOVA with repeated measures: F(2,22) = 1.43, P > 0.1).

Discussion
The results show, in agreement with a number of previous reports [6, 7, 10, 11], that both noradrenaline and phenylephrine constrict the dorsal hand vein in a reproducible dose-dependent manner. Furthermore, noradrenaline appeared to be more potent than phenylephrine, its log E_max value being approximately forty times smaller than that of phenylephrine. This finding is consistent with previous reports showing that noradrenaline is a more potent venoconstrictor both in the human dorsal hand vein [6] and in the human isolated femoral vein [22].

Both oral doses (5 and 10 mg) of BIS could antagonize the venoconstrictor responses to noradrenaline and phenylephrine leading to rightward shifts in the dose-response curves and increases in the values of E_max. The antagonism was dose dependent. This was a surprising finding since the venoconstrictor responses are known to be mediated by the activation of α₁-adrenoceptors: noradrenaline can activate both venoconstrictor a₁- and a₂-adrenoceptors, and...
Bisoprolol and dorsal hand vein

Noradrenaline

Phenylephrine

Figure 3 Parameters of the dose-response curves (a: log E\text{D}_50, b: geometric mean, c: E\text{max}), to noradrenaline (upper panel) and phenylephrine (lower panel), (n = 12), calculated from individual subject data, 2 h after oral ingestion of placebo (open), bisoprolol 5 mg (hatched), and bisoprolol 10 mg (closed).

Table 2 Parameters of dose-response curves: differences (mean, 95% CI) between placebo (Pl) and bisoprolol 5 mg (BIS5), and bisoprolol 10 mg (BIS10).

<table>
<thead>
<tr>
<th></th>
<th>Log E\text{D}_50</th>
<th>E\text{max}</th>
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<tbody>
<tr>
<td>Noradrenaline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl/BIS5</td>
<td>−0.32 (−0.57, −0.06)</td>
<td>14.71 (−2.67, 32.09)</td>
</tr>
<tr>
<td>Pl/BIS10</td>
<td>−0.53 (−0.78, −0.28)</td>
<td>5.27 (−12.11, 22.21)</td>
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<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
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<tr>
<td>Pl/BIS5</td>
<td>−0.40 (−0.15, −0.65)</td>
<td>−2.71 (−20.13, 14.27)</td>
</tr>
<tr>
<td>Pl/BIS10</td>
<td>−0.59 (0.34, −0.84)</td>
<td>−2.39 (−19.77, 14.99)</td>
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</table>

Table 3 Cardiovascular measures.

<table>
<thead>
<tr>
<th></th>
<th>Change from pretreatment baseline (mean ± s.e. mean)</th>
<th>Difference from placebo (mean (95% CI))</th>
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</thead>
<tbody>
<tr>
<td>Heart rate (beats min\text{^-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>−3.58 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>Bisoprolol 5 mg</td>
<td>−10.67 ± 1.68*</td>
<td>7.10 (3.87, 10.33)</td>
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<tr>
<td>Bisoprolol 10 mg</td>
<td>−15.25 ± 1.75*</td>
<td>11.67 (8.44, 14.90)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>−0.83 ± 1.52</td>
<td></td>
</tr>
<tr>
<td>Bisoprolol 5 mg</td>
<td>−8.08 ± 1.66*</td>
<td>7.22 (3.28, 11.22)</td>
</tr>
<tr>
<td>Bisoprolol 10 mg</td>
<td>−10.08 ± 1.71*</td>
<td>9.25 (5.28, 13.22)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>−0.67 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>Bisoprolol 5 mg</td>
<td>1.83 ± 1.11</td>
<td>1.17 (−1.66, 4.00)</td>
</tr>
<tr>
<td>Bisoprolol 10 mg</td>
<td>−3.33 ± 1.12</td>
<td>2.67 (−0.14, 5.50)</td>
</tr>
</tbody>
</table>

*P < 0.05 (Analysis of variance, followed by Dunnett’s test).

Phenylephrine is a selective \(\alpha_1\)-adrenoceptor agonist. It is known that some \(\beta\)-adrenoceptor antagonists such as labetalol and carvedilol have affinity for \(\alpha_1\)-adrenoceptors and thus can antagonize \(\alpha_1\)-adrenoceptor mediated vasoconstrictor responses [23–27]. However, BIS is a highly selective \(\beta_1\)-adrenoceptor antagonist with no affinity for either \(\alpha_1\)- or
\[ \pi_2 \text{-adrenoceptors} \text{[13, 14], and thus an interaction with } \pi_2 \text{-adrenoceptors cannot explain the present observations.} \]

Another possible mechanism to be considered is a partial agonistic activity of BIS at \[ \beta_2 \text{-adrenoceptors} \text{(the so-called intrinsic sympathomimetic activity, ISA), which has been shown to be the characteristic of some } \beta_2 \text{-adrenoceptor antagonists, for example expropranolol [10]. The operation of such a mechanism would lead to functional antagonism between } \alpha \text{-adrenoceptor mediated vasoconstriction and } \beta_2 \text{-adrenoceptor mediated vasodilatation. In the case of functional antagonism two agonists act at two receptor systems mediating opposite effects via the same effector system. This drug-receptor interaction can lead to a shift in the dose-response curve to one of the agonists in the presence of the other agonist which is indistinguishable from competitive antagonism [29, 29]. However, again, there is no evidence indicating that BIS has any agonistic activity at either } \beta_1 \text{- or } \beta_2 \text{-adrenoceptors [13, 14].} \]

Therefore it is unlikely that the antagonism of the venoconstrictor responses to noradrenaline and phenylephrine by BIS is due to an interaction at adrenoceptors. A further possibility is that the \[ \beta_2 \text{-adrenoceptor antagonist} \] might have caused venodilatation by a direct smooth muscle relaxant effect. Although it has been shown that some \[ \beta_2 \text{-adrenoceptor antagonists} \text{(e.g. SK&F 92657) may have some such hyaluranes-like effect in the dorsal hand vein [30], there is evidence against such an action of BIS [31].} \]

It is of interest to consider the possibility that an endothelium-related mechanism is involved in the antagonistic effects of BIS. Indeed it has been reported recently that the selective \[ \beta_2 \text{-adrenoceptor antagonist nebivolol can dilate the human dorsal hand vein [11] due to its ability to release nitric oxide from vascular endothelium [11, 12]. It remains to be shown whether the same mechanism operates in the case of BIS. Furthermore, it has been reported that chronic administration of BIS leads to the inhibition of the synthesis of the venoconstrictive substance endothelin-1 [32]. However, it is unlikely that an effect on endothelin-1 generation is involved in our observation since it has been shown that the human dorsal hand vein has no intrinsic basal tone [33, 34].} \]

In agreement with its well documented cardiovascular effects, the single oral dose of BIS caused significant decreases in systolic blood pressure and heart rate [35–37]. These observations are consistent with the blockade of cardiac \[ \beta_1 \text{-adrenoceptors.} \] It should be pointed out, however, that it is unlikely that a reduction in systemic arterial blood pressure could have led to a reduction in the size of the venoconstrictor responses to noradrenaline and phenylephrine in the dorsal hand vein since the degree of the venodilatation between the applications of the agonist was kept constant by using a standard (45 mmHg) congestion pressure. Indeed we have found that the initial venous diameter was not affected by the ingestion of BIS (see Table 1), and it has been reported that propranolol, a \[ \beta_2 \text{-adrenoceptor antagonist} \] which also lowers systemic blood pressure, has no effect on the diameter of the dorsal hand vein [4].

The present findings also have bearing on the possible role of \[ \beta_2 \text{-adrenoceptors} \] in the dorsal hand vein. Although the existence of venodilator \[ \beta_2 \text{-adrenoceptors} \] is well documented [38, 39], the possible involvement of \[ \beta_2 \text{-adrenoceptors} \] in mediating venodilator effects is more controversial [4, 41]. In an early study White & Udwadia [4] reported that both the non-selective \[ \beta_2 \text{-adrenoceptor antagonist} \] propranolol and the selective \[ \beta_2 \text{-adrenoceptor antagonist} \] practolol can potentiate the constrictor response of the dorsal hand vein to noradrenaline. This observation can be interpreted as evidence for the blockade of masked venodilator \[ \beta_2 \text{-adrenoceptors} \] by the antagonists resulting in an increase in the size of the constrictor response [40]. The effectiveness of practolol would suggest that \[ \beta_2 \text{-adrenoceptors} \] are involved in mediating the venodilator effect of noradrenaline. The potentiation of the constrictor responses to noradrenaline by practolol is a surprising finding since practolol has pronounced partial agonistic activity [31] which is expected to lead to venodilatation which in turn could counteract the potentiation of the venoconstriction resulting from \[ \beta_2 \text{-adrenoceptor blockade.} \] Furthermore, if noradrenaline activated masked inhibitory \[ \beta_2 \text{-adrenoceptors}, \] we would have expected that in our experiment the response to noradrenaline would have been potentiated whereas the response to phenylephrine, which has little affinity for \[ \beta_2 \text{-adrenoceptors,} \] would have remained unaffected. However, as responses to both noradrenaline and phenylephrine were antagonized by BIS, we are unable to confirm the existence of venodilator \[ \beta_2 \text{-adrenoceptors in the dorsal hand vein.} \] In this respect it would be of interest to examine the effects of atenolol, another selective \[ \beta_2 \text{-adrenoceptor antagonist,} \] on venoconstrictor responses to noradrenaline in the human dorsal hand vein since it has been shown that this drug has no effect on venoconstrictor responses to phenylephrine [11].

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References

1. Breidte O-E, Zerkowski H-K, Doetch N, Khamsi M. Subepicardial up-regulation of human saphenous vein \[ \beta_2 \text{-adrenoceptors} \] by chronic \[ \beta_2 \text{-adrenoceptor antagonist} \] treatment. Nanotroph-Schmidtberg’s Arch Pharmacol 1989; 339: 479–482.

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