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Influence of Carvedilol on the Responsiveness of Human Hand Veins to Noradrenaline and Dinoprost

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Carvedilol is a new agent with β-blocking and vasodilating properties. This has been shown in both human and animal studies (Strein et al. 1987; von Möllendorff et al. 1986). Until now, the mechanism of carvedilol-induced vasodilatation has not been completely clarified. Carvedilol has been found to possess α_1-antagonistic properties (Cubeddu et al. 1987), but animal investigations have suggested mechanisms independent of α_1-blockade (Sponer et al. 1987, 1988).

Challenges with agonists have been found to be extremely useful for testing the mechanism of action of antagonistic drugs. β-Blocking activity can, for example, be titrated by use of isoprenaline (Wellstein et al. 1988). One problem with some agonist challenges is undesired systemic effects, which may limit the use of these methods. Aellig (1981a,b) previously described a method for avoiding these problems. With this method the influence of very low and therefore not systemically active doses of noradrenaline and other agonists on hand veins is easily established. The agonists are infused into a human hand vein which is constricted at a pressure of 40mm Hg. By use of this method, nearly complete dose-effect curves can be derived *in vivo* without systemic effects. The influence of compounds with unknown mechanisms of action on those dose-effect curves can then be tested.

In the present study, noradrenaline was used as a specific α-agonist and dinoprost (prostaglandin $F_2\alpha$) as a non-adrenergic vasoconstrictor with a mechanism of action which is not dependent on the sympathetic system (Robinson et al. 1973). The aim of the present study was to establish the influence of carvedilol on the dose-effect curves of these substances and thus gain further insight into the mechanism of action of carvedilol.

1. Methods

Eight healthy male volunteers (aged 23 to 30 years and weighing 72 to 84kg) participated in the study after their fully informed written consent had been obtained. The study was performed according to the ethical principles embodied in the Declarations of Helsinki and Tokyo.

The single-blind study followed a 4-way randomised crossover design. Each volunteer received oral doses of 50mg of carvedilol twice and placebo twice, with washout periods of more than 48 hours between the 4 study days.

Noradrenaline (Arterenol® 1 mg/ml, Hoechst, FRG) was diluted with sterile saline to a concentration of 120 ng/ml and vitamin C 4 mg/ml was added as an antioxidant. The solution was infused continuously with a Perfusor ED 2 (Braun, Melsungen) at the following dosages: 6, 18, 54, 162 and 486 ng/min, in volumes of from 3 to 243 ml/h. Each dose step was given over 4 minutes.

Dinoprost (5 mg/ml, Upjohn, Heppenheim) was diluted in the same way, but no vitamin C was added. The dosages given were 200, 600, 1800 and 5400 ng/min in volumes of from 3 to 81 ml/h. Each dose step was terminated after 4 minutes.

Table I. Diameter of the hand veins (mm) during 40mm Hg occlusion of the upper arm (cuff) and during infusion of saline at 1.6 ml/min (mean ± SEM; n = 8)

	Placebo		Carvedilol	
	noradrenaline	dinoprost	noradrenaline	dinoprost
Before medication	1.30 ± 0.12	1.34 ± 0.10	1.34 ± 0.08	1.33 ± 0.07
1 hour after oral medication	1.27 ± 0.11	1.32 ± 0.10	1.29 ± 0.09	1.25 ± 0.06
3.5 hours after oral medication	1.28 ± 0.11	1.27 ± 0.10	1.30 ± 0.09	1.27 ± 0.06

1.1 Protocol

Volunteers reported to the laboratory after an overnight fast and remained fasting until the completion of each study day. With the patient in the supine position, a hand was elevated above heart level and a superficial hand vein which showed no crossing(s) with other veins was punctured with a butterfly needle (25G). This needle was kept patent during the whole experiment by an infusion of 0.9% physiological saline at a rate of 0.83 ml/min, except for that period when the vasoconstrictors were infused. One hour after the baseline vasoconstrictor dose-effect curve had been obtained, the subjects received carvedilol 50mg or placebo orally with 100ml of water, and 1 hour and 3.5 hours after drug intake the vasoconstrictor dose-effect curves were repeated.

1.2 Vasoconstrictor Dose-Effect Curves

According to the method of Aellig (1981a,b), the diameter of the hand vein (represented by the change in skin level above the summit of the vein), was measured by use of a Schaewitz receptor fitted to an amplifier (Venograph Boucke, Tübingen, FRG) so that a chart registration of the diameter of the hand vein could be received. A cuff was placed around the upper arm, allowing a venous occlusion pressure of 40mm Hg to be maintained. The cuff was inflated only for the last 2 of the 4 minutes taken for each dose step, thus allowing the hand vein to deflate between measurements.

In contrast to Aellig's method the dosage was increased in this study by increasing the infusion rate at a constant concentration of the agonist. To exclude any influence by the infused volume, saline was infused at a rate of 1.6 ml/min at the baseline of each dose-effect curve. The hand vein response to these conditions was not influenced by the volume and thus served as a 100% vein distension for the dose-effect curves.

The diameter of the hand vein was measured as the maximum response was reached at the end of the 2-minute occlusion at 40mm Hg and compared with the deflated state before and after the dose steps.

Table II. Carvedilol plasma concentrations (ng/ml) as mean (± SEM) of the concentrations measured before and at the end of the noradrenaline and dinoprost dose-effect curves in the hand vein (n = 8)

	Noradrenaline dose-effect curve	Dinoprost dose-effect curve
1 hour	59.9 ± 21.0	67.9 ± 23.0
3.5 hours	53.8 ± 19.6	48.6 ± 16.0

1.3 Data Evaluation

Results of measurements from the separate experiments were calculated as a percentage reduction in hand vein diameter and were then pooled to give mean ± SEM.

To exclude interindividual differences as regards responsiveness to the vasoconstrictors, the following approach was used: data were evaluated with a Hewlett Packard desk top calculator (model 9836) and a program package for non-linear least

square curve fitting (GIP, Giessen iteration procedure; Wiemer et al. 1982). For each individual the dose-response curve measured before administration of carvedilol was fitted to the data using the following equation:

$$E_A = E_{max}/1 + (A/EC_{50})^{nH}$$

where E_A denotes the effect, E_{max} the maximal capacity of the effect, A the dose of the vasoconstrictor during infusion and nH the slope of the curve. From these curves the doses of the vasoconstrictors which induced a 30% and a 60% reduction in hand vein diameter, respectively, were determined. The dose-response curves obtained 1 hour and 3.5 hours after carvedilol administration were calculated by use of the same procedure. By the use of these results, the degree of vasoconstriction at that concentration of noradrenaline and dinoprost which yielded a 30% and a 60% vasoconstriction in the control curve was obtained.

1.4 Carvedilol Plasma Concentrations

Before the start and at the end of each of the dose-effect curves, blood was withdrawn and plasma sampled for estimation of the carvedilol plasma concentrations. The method for drug analysis employs an internal standard, liquid-liquid extraction, re-extraction and reversed-phase high performance liquid chromatography (HPLC) with fluorometric detection (Reiff 1987). The detection limit was 1 ng/ml.

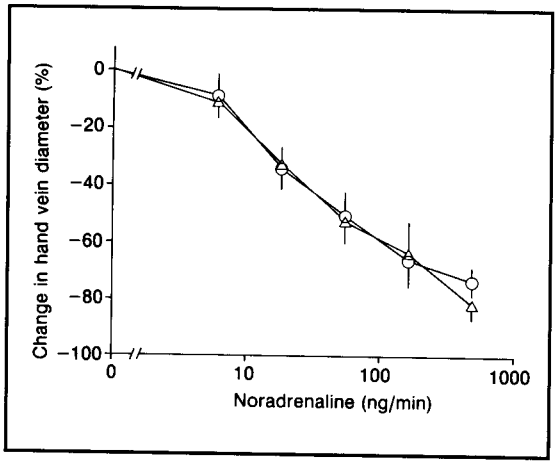

Fig. 1. Dose-response curve for the vasoconstrictor effect of noradrenaline during infusion into superficial hand veins before (○) and 1 hour after (△) oral administration of placebo (0% = diameter before vasoconstriction; 100% = theoretical complete vasoconstriction); mean ± SEM (n = 8).

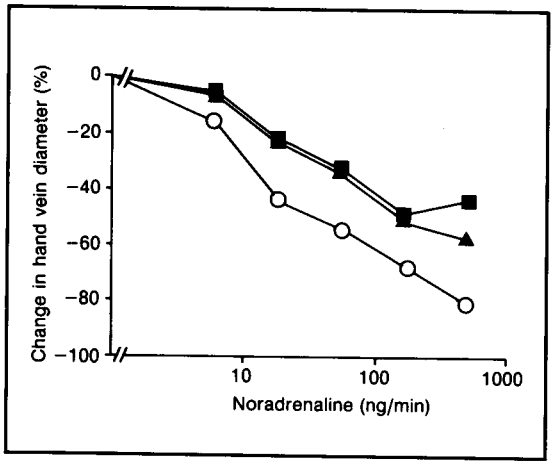

Fig. 2. Dose-response curve for the vasoconstrictor effect of noradrenaline during infusion into superficial hand veins before (○) and 1 hour (■) and 3.5 hours (▲) after oral administration of carvedilol 50mg (0% = diameter before vasoconstriction; 100% = theoretical complete vasoconstriction); mean ± SEM (n = 8).

2. *Results*

Table I shows the mean hand vein diameters during infusion of saline before vasoconstrictor infusions. It is obvious that neither placebo nor carvedilol significantly change the hand vein diameter at 40mm Hg and that the method shows a good reproducibility. In addition, the mean concentrations of carvedilol are presented in table II; the variability is mainly an interindividual one.

2.1 Noradrenaline

Noradrenaline produced a dose-dependent reduction in the diameter of the hand vein; the reproducibility of the method can be seen from figure 1, which shows an unchanged response before, and 1 hour after, an oral placebo. The same reproducibility was also seen 3.5 hours after placebo

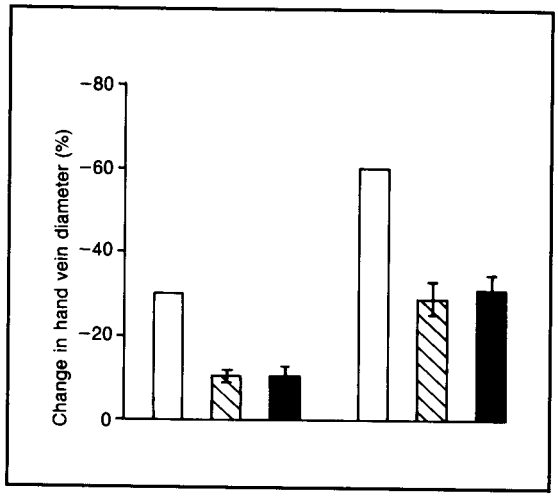

Fig. 3. Reduction of hand vein diameter by noradrenaline before (□) and 1 hour (▨) and 3.5 hours (■) after oral administration of carvedilol 50mg (for details see section 1.3); mean ± SEM (n = 8).

and from a comparison of the mean day to day response to noradrenaline before the oral medications had been given (unpublished data). The ED_{50} in the control experiments without carvedilol was approximately 43 ng/min. Carvedilol clearly shifted the noradrenaline dose-effect curve to the right at both 1 and 3.5 hours (fig. 2). The noradrenaline doses which before carvedilol intake induced a 30% and a 60% reduction of the vein diameter, at 1 hour and 3.5 hours yielded only a 10% and a 29% and an 11% and a 30% effect, respectively (fig. 3).

2.2 Dinoprost

Although dinoprost has a lower potency than noradrenaline, this vasoconstricting prostaglandin also produced a dose-dependent reduction in the diameter of the hand vein. The reproducibility of this response (fig. 4) was identical to that of noradrenaline. The ED_{50} values in the control experiments were approximately 1175 ng/min. The reproducibility of the effect of dinoprost is shown in figure 4, which demonstrated nearly identical dose-effect curves before, and 1 hour after, the oral administration of placebo.

Dinoprost dose-effect curves 1 and 3.5 hours after carvedilol administration are shifted to the right (fig. 5). The doses which before carvedilol intake induced a 30% and a 60% reduction of the vasoconstriction, at 1 hour and 3.5 hours yielded only a 6% and a 12% and an 18% and a 27% response, respectively (fig. 6). This effect diminished after 3.5 hours, which contrasts to the unchanged noradrenaline effects on the hand vein (fig. 3).

3. Discussion

This study clearly demonstrated the reliability of Aellig's method for establishing *in vivo* dose-effect curves of vasoconstricting substances in the human hand vein. This could be proven for both noradrenaline and dinoprost. The method shows an excellent reproducibility and provides a unique chance for establishing *in vivo* dose-effect curves in man that cover the complete response from the minimum to the maximum effect, without inducing systemic effects.

It is obvious from the dose-effect curves shown, as well as from the more specific analysis of the

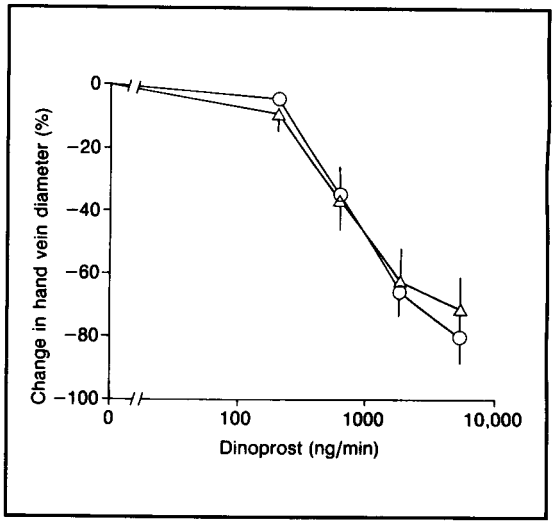

Fig. 4. Dose-response curve for the vasoconstrictor effect of dinoprost during infusion into superficial hand veins before (○) and 1 hour after (△) oral administration of placebo (0% = diameter before vasoconstriction; 100% = theoretical complete vasoconstriction); mean ± SEM (n = 8).

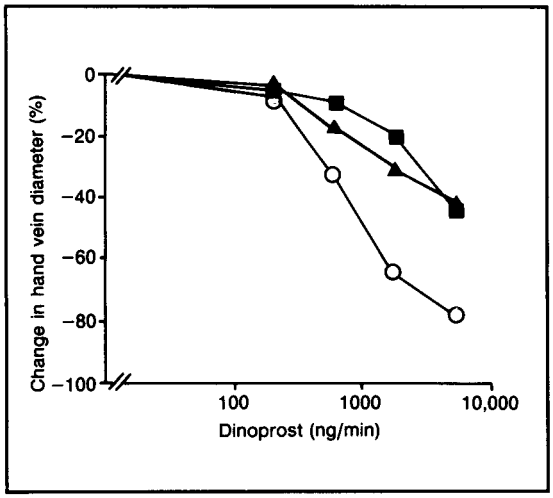

Fig. 5. Dose-response curve for the vasoconstrictor effect of dinoprost during infusion into superficial hand veins before (○) and 1 hour (■) and 3.5 hours (▲) after oral administration of carvedilol 50mg (0% = diameter before vasoconstriction; 100% = theoretical complete vasoconstriction); mean ± SEM (n = 8).

response to noradrenaline and dinoprost doses which induced graded effects (30% and 60%), that both the response to a specific α-agonist (noradrenaline) and the response to a vasoconstrictor which does not exert its effects via the sympathetic nervous system were attenuated by carvedilol. One hour after the administration of this β-blocker with vasodilating properties, the effects of the prostaglandin-type vasoconstrictor were even more attenuated than those of the sympathomimetic drug. This implies that this new compound, beside its well-proven β-blocking activity and α_1-antagonistic properties, possesses additional anti-vasoconstrictive properties, which, 1 hour after administration, are more pronounced than its effect on the α-sympathetic system. The following limitations need to be considered when interpreting these experiments:

- In these studies only single doses of carvedilol were used. During long term therapy the situation may be different.
- These results were derived from human hand veins *in vivo*. Although these can be considered to give distinctly more reliable insight into clinically relevant mechanisms than many animal experiments, the behaviour of blood pressure and the arterial circulation may be different. However, it is known that in general, there are only small differences in the response to the vasoconstrictors used between the vascular smooth muscle of the human arterial and venous circulation (Robinson et al. 1973).
- At present, no controlled data allowing clear statements on the effects of other vasodilators and/or sympatholytic agents after systemic administration on the dose-effect curves of noradrenaline and dinoprost are available.

4. Conclusions

These experiments prove that carvedilol 50mg does not only attenuate the vasoconstrictive effect of noradrenaline on the human hand vein but also diminishes that of a vasoconstrictor which does not exert its effects via the sympathetic nervous system.

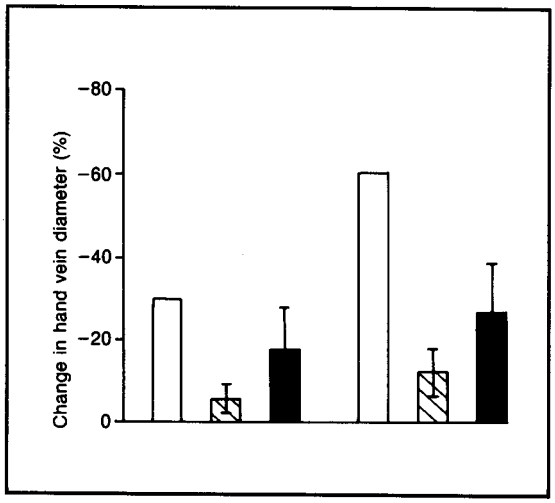

Fig. 6. Reduction of hand vein diameter by dinoprost before (□) and 1 hour (▨) and 3.5 hours (■) after oral administration of carvedilol 50mg (for details see section 1.3); mean ± SEM (n = 8).

Acknowledgement

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References

Aellig WH. A new technique for recording compliance of human hand veins. British Journal of Clinical Pharmacology 11: 237-243, 1981a

Aellig WH. Pharmacological experiments on human hand veins *in situ*. International Journal of Clinical Pharmacology Research 1: 103-109, 1981b

Cubeddu LX, Fuenmayor N, Varin F, Villagra VG, Colindres RE, et al. Clinical pharmacology of carvedilol in normal volunteers. Clinical Pharmacology and Therapeutics 41: 31-44, 1987

Reiff K. High-performance liquid chromatographic method for the determination of carvedilol and its desmethyl metabolite in body fluids. Journal of Chromatography 413: 355-362, 1987

Robinson BF, Collier JG, Karim SMM, Somers K. Effect of prostaglandins A_1, A_2, B_1, E_2 and F_2 on forearm arterial bed and superficial hand veins in man. Clinical Science 44: 367-376, 1973

Sponer G, Strein K, Müller-Beckmann B, Bartsch W. Studies on the mode of vasodilating action of carvedilol. Journal of Cardiovascular Pharmacology 10 (Suppl. 11): S42-S48, 1987

Sponer G, Bartsch W, Strein K, Müller-Beckmann B, Kling L. Mode of vasodilating activity of carvedilol in isolated perfused hind limbs of rabbits. Drugs 36 (Suppl. 6): 55-61, 1988

Strein K, Sponer G, Müller-Beckmann B, Bartsch W. Pharmacological profile of carvedilol, a compound with β-blocking and vasodilating properties. Journal of Cardiovascular Pharmacology 10 (Suppl. 11): S33-S41, 1987

von Möllendorff E, Abshagen U, Akpan W, Neugebauer G, Schröter E. Clinical pharmacologic investigations with carvedilol, a new β-blocker with direct vasodilator activity. Clinical Pharmacology and Therapeutics 39: 677-682, 1986

Wellstein A, Belz GG, Palm D. β-Adrenoceptor subtype binding activity in plasma and β-blockade by propranolol and β_1-selective bisoprolol in humans. Evaluation by Schild-plots. Journal of Pharmacology and Experimental Therapeutics 246: 328-337, 1988

Wiemer G, Wellstein A, Palm D, von Hattingberg HM, Brockmeier D. Properties of agonist binding at the β-adrenoceptor of the rat reticulocyte. Naunyn-Schmiedeberg's Archives of Pharmacology 321: 11-19, 1982

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