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The effect of prazosin on skin microcirculation as assessed by laser Doppler flowmetry

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1 Laser Doppler flowmetry was used in six normal volunteers to record changes in fingertip skin blood flow after the administration of prazosin to block postsynaptic α_1-adrenoceptors.

2 Prazosin (0.5 mg orally) did not alter systolic or diastolic blood pressure or heart rate.

3 Prazosin did significantly increase basal skin blood flow 2 h after its administration but this effect was no longer evident after contralateral hand warming. Prazosin markedly reduced the skin vasoconstrictor response to deep inspiration and to contralateral hand cooling.

4 This study suggests that postsynaptic α_1-adrenoceptors are involved in maintaining skin vasoconstrictor tone at rest and are also involved in the rapid skin vasoconstriction seen in response to a deep inspiration and to contralateral hand cooling.

Keywords α-adrenoceptor blockers microcirculation flowmeters prazosin lasers

Introduction

The peripheral cutaneous microcirculation is thought to be under dual control, centrally by neural regulation and locally by myogenic tone and by metabolites which accumulate in ischaemic tissue and cause vasodilatation (Arnott & MacFie, 1948). From animal studies, the principal neural regulator of skin vasoconstriction would appear to be the sympathetic nervous system (SNS) (Hales *et al.*, 1982; Molyneux & Hales, 1982). In fact, this SNS vasconstriction appears to be extremely potent such that it is even able to override maximal levels of locally mediated vasodilatation (Johnson *et al.*, 1976).

Further study of the role of skin SNS activity in man has been hampered by methodological limitations. This is because small blood vessels inevitably have a narrow synaptic cleft and this causes there to be a rapid and transient response to neuronally released noradrenaline (NA) (Bevan, 1987). Most previous methods of investigating the microcirculation *in vivo* in man did not have a rapid enough response time, to record this phenomenon accurately. This limitation was particularly true when an attempt was made to study the rapid transient vasoconstriction which occurs as a result of deep inspiration or contralateral hand cooling. The recent advent of laser Doppler flowmetry with its rapid response time (0.2 s) now allows us to overcome this methodological problem (Stern, 1975).

The laser Doppler technique has already proven useful in investigating the skin microcirculation in man (Stern *et al.*, 1977; Sundberg, 1984). We now report on its use to investigate the role of the SNS in the skin microcirculation with particular reference to the rapid vasoconstriction which occurs in response to deep inspiration (Bolton *et al.*, 1936) and contralateral hand cooling. We used prazosin as a postsynaptic α_1-adrenoceptor antagonist to examine whether these receptors were involved in the above manoeuvres.

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Methods

The study was performed on six normotensive male subjects with a mean age of 25 ± 5 years and a mean weight of 69.5 ± 12.3 kg (mean ± s.d.). Subjects were excluded if they showed any significant abnormalities in clinical examination, an electrocardiogram or routine laboratory indices. All subjects gave their informed consent to the study which was approved by the local Ethics Committee.

Subjects arrived fasting on the morning of the experiment and rested for 20 min. They were seated comfortably for 2.5 h with the left arm resting and supported on a table top at heart level. The studies were performed in a room with a controlled ambient temperature of 25 ± 1 °C. Subjects attended on two occasions at least 1 week apart. On both days, core temperature was measured initially and the study only proceeded if this was in the normal range (37 ± 0.8 °C). On one day they were given oral prazosin (0.5 mg) and on the other a matching placebo tablet in a randomised single-blind fashion. On each study day they underwent the following tests before, at 1 h and 2 h after drug administration.

Finger tip blood flow was recorded for 10 min (FTBFeq) followed by warming of the right hand (contralateral hand warming) for 10 min in water maintained at 43° C. This warming was employed to reduce oscillations in FTBF due to vasomotion associated with spontaneous bursts of skin sympathetic activity (Bini *et al.*, 1980). This produced a centrally mediated general vasodilatation and relatively stable FTBF trace, although some oscillation is still seen in the trace due to the pulse pressure from each heart beat (Figure 2). From this trace a value for the prestimulus FTBF after warming (FTBFw) was estimated for each subject. At this point blood pressure, heart rate and finger tip temperature were also measured.

Reflex vasoconstriction was assessed at 0, 1 and 2 h postdrug by an inspiratory gasp (Johnson & Spalding, 1974) and a cold challenge as previously employed (Emslie-Smith *et al.*, 1986). The inspiratory gasp consisted of a sudden deep breath and the cold challenge was performed by transferring the right hand from the warm water (43° C) to cold water (15 °C) for 2 min. The temperature of the cold water is considerably warmer than a standard cold pressor test where iced water at 4° C is commonly used to cause a pressor response and an increase in plasma noradrenaline. Indeed blood pressure and heart rate did not change in response to cold water in this study (Table 1). The normal microcirculatory response to an inspiratory gasp or to cold immersion at 15° C is rapid transient vasoconstriction with a decrease in FTBF which quickly returns to its original level (Figure 2). This vasoconstriction is thought to be mediated by a spinal reflex with a sympathetic efferent pathway. A quantitative index of this vasoconstrictor reflex (VR) was determined using the expression:

$$VR = \frac{FTBFw - FTBFmin}{FTBFw}$$

where FTBFmin is the minimum blood flow recorded following the inspiratory gasp and cold challenge and FTBFw is as previously described.

Blood pressure, heart rate and finger tip temperature were also measured during cold immersion.

Core temperature was measured at the beginning of the study only by a zero gradient aural thermometer (Addison Process Control Ltd, type 8151.1) (Keating & Sloan, 1975). Finger tip (middle finger left hand) temperature was monitored throughout with a skin thermistor (YSI Model No. 4098). Blood pressure was monitored with a manual sphygmomanometer by an observer who was unaware of the treatments given. Heart rate was measured by palpation of the radial pulse. The ECG was continuously monitored with a Hewlett Packard monitor (model 78351 A).

Cutaneous finger tip blood flow (FTBF) was continuously recorded from the index finger of the left hand using a laser Doppler flowmeter (Model PF2B, Perimed, Stockholm Sweden). The principles governing the measurement of skin blood flow by this technique have been described in detail elsewhere (Nilsson *et al.*, 1980). Essentially, light from a 2 mW helium-neon laser is brought to the skin surface by an optical fibre and undergoes multiple scattering and absorption in a small hemispherical volume of tissue with a radius of approximately 1 mm (Holloway, 1983). The light beams scattered by moving erythrocytes undergo a frequency shift (Doppler effect) and the back-scattered light is picked up by efferent optical fibres in the head of the probe and taken back to the instrument for signal processing. The Doppler-shifted portion of the signal is converted into a voltage output signal which is linearly related to the product of the number of red blood cells and their mean velocities in the measuring volume. The output signal was recorded on a pen recorder (Full scale = 10 V, gain × 3).

Statistical analysis

Results are presented as means ± s.d. The effect of prazosin was assessed by calculating the

changes from baseline at 1 h (or 2 h) after prazosin and comparing this with the corresponding change from baseline 1 h (or 2 h) after placebo. For this comparison, a paired *t*-test was employed and a level of $P < 0.05$ was taken as indicating statistical significance.

Results

No significant changes occurred in blood pressure or heart rate throughout the study with either placebo or prazosin. Blood pressure and heart rate did not change significantly at any time following cold challenge (Table 1).

Finger tip temperature was not significantly different during the equilibrium period with placebo or prazosin throughout the study. Following contralateral hand warming finger tip temperature rose as FTBF increased. There was no significant difference in finger tip temperature prior to the cold challenge with the placebo or prazosin throughout the experiment (Table 1).

Before contralateral hand warming equilibrium finger tip blood flow (FTBFeq) was not significantly different between the placebo and prazosin day at 1 h after administration (Figure 1). The FTBFeq was however significantly higher with prazosin ($P < 0.05$) 2 h after administration (Figure 1).

Following contralateral hand warming the pre-stimulus finger tip blood flow ($FTBF_w$) increased with placebo and prazosin. The level of FTBF achieved by this method was not significantly different between prazosin and placebo at 1 h or 2 h after administration (Figure 1).

Figure 2 shows a representative VR response following inspiratory gasp and cold challenge. Figure 2 also shows the oscillation due to pulse pressure which remains even after contralateral hand warming. The VR following inspiratory gasp was not significantly different between placebo and prazosin 1 h after administration. It fell significantly with prazosin ($P < 0.05$) 2 h after administration (Figure 3). The VR following cold challenge fell significantly ($P < 0.05$) with prazosin at both 1 h and 2 h after administration.

Discussion

Laser Doppler flowmetry is a relatively new technique which is based on the fact that moving red blood cells cause a frequency shift (Doppler effect) when they scatter back a laser light beam (Stern, 1975). The size of the Doppler signal therefore reflects the amount of red cell movement within the tissue. Its great advantage over the other methods of studying the microcirculation such as ^{133}Xenon clearance or photoplethysmography is that it gives a continuous signal which responds rapidly to vascular perturbation. The major disadvantage about laser Doppler flowmetry is that it is difficult to express its output quantitatively. This is especially true of its recording of basal blood flow since this is often characterised by irregular rhythmic oscillations. These oscillations are due to two phenomena: spontaneous sympathetic activity and pulse pressure. Contralateral hand warming abolishes the former but not the latter (Figure 2).

In this study, we have simply averaged the

Table 1 The effect of placebo or prazosin (0.5 mg) on blood pressure, heart rate and finger tip temperature in six normal volunteers. Prazosin had no significant effect on any of above parameters

	Placebo			*Prazosin*		
	Before	*1 h*	*2 h*	*Before*	*1 h*	*2 h*
Systolic pressure (mmHg)	115 ± 18	111 ± 18	116 ± 18	112 ± 11	106 ± 15	105 ± 15
Diastolic pressure (mmHg)	71 ± 8	74 ± 10	73 ± 9	66 ± 9	64 ± 9	63 ± 7
Heart rate (beats min^{-1})	67 ± 7	66 ± 11	66 ± 13	65 ± 9	67 ± 10	66 ± 9
Finger tip temperature (°C)	30 ± 2.6	30.1 ± 2.8	30.6 ± 1.4	31.2 ± 0.9	31.1 ± 1.6	31.5 ± 1.0
After contralateral hand warming						
Systolic pressure (mmHg)	113 ± 17	111 ± 16	111 ± 15	108 ± 16	106 ± 16	110 ± 16
Diastolic pressure (mmHg)	72 ± 8	73 ± 8	70 ± 11	67 ± 8	63 ± 5	65 ± 7
Heart rate (beats min^{-1})	71 ± 8	67 ± 13	67 ± 12	69 ± 9	70 ± 11	72 ± 8
Finger tip temperature (°C)	32 ± 1.3	31.9 ± 0.7	32.2 ± 0.8	32 ± 0.7	32.1 ± 0.6	32.1 ± 0.7
After contralateral hand cooling						
Systolic pressure (mmHg)	112 ± 15	114 ± 16	113 ± 17	108 ± 17	107 ± 16	109 ± 17
Diastolic pressure (mmHg)	71 ± 8	74 ± 9	70 ± 9	67 ± 9	64 ± 6	65 ± 8
Heart rate (beats min^{-1})	70 ± 7	69 ± 11	68 ± 12	70 ± 8	72 ± 10	69 ± 9
Finger tip temperature (°C)	31.7 ± 1.6	31.9 ± 1.0	31.9 ± 0.8	31.7 ± 0.7	31.9 ± 0.6	31.7 ± 0.7

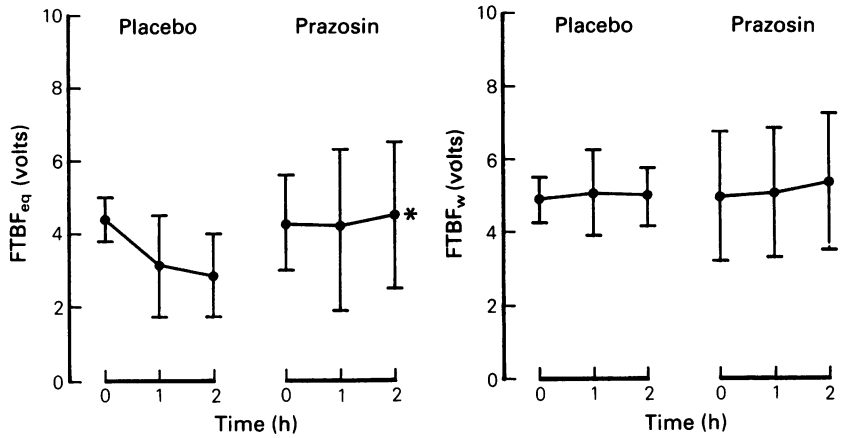

Figure 1 Finger tip blood flow at equilibrium (FTBFeq) and after contralateral hand warming (FTBFw) in six normal volunteers before and after placebo or prazosin (0.5 mg). * $P < 0.05$ comparing the effect of prazosin from the effect of placebo at equivalent times.

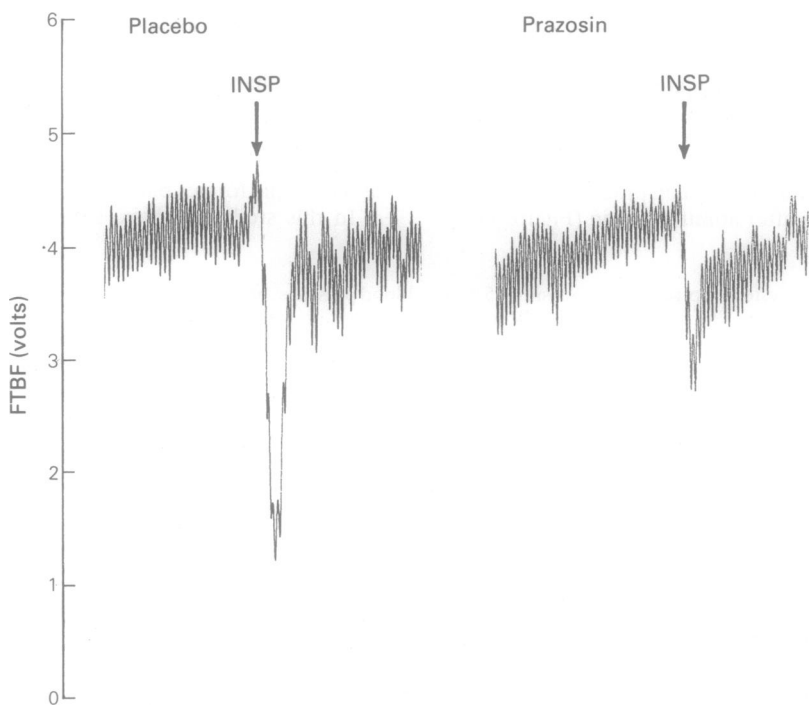

Figure 2 Representative laser Doppler flowmetry trace during an inspiratory gasp after placebo or prazosin (0.5 mg). The oscillations seen in the resting trace are due to pulse pressure.

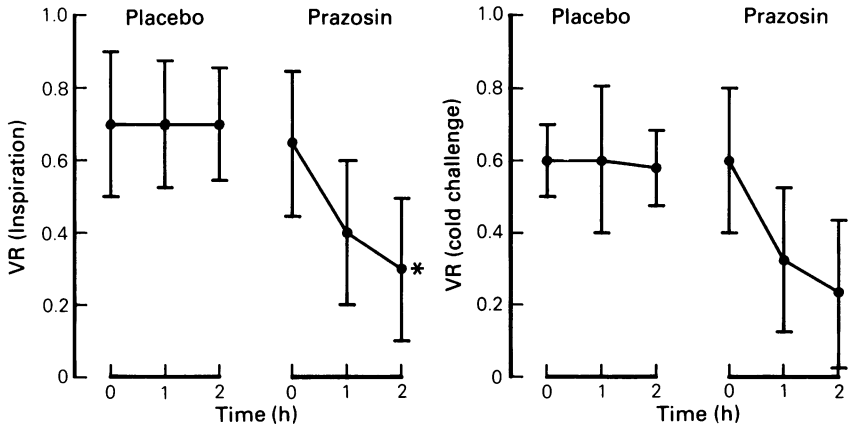

Figure 3 Vasoconstrictor reflex after inspiration and cold challenge in six normal volunteers before and after placebo or prazosin (0.5 mg) * $P < 0.05$ comparing the effect of prazosin and the effect of placebo at equivalent times.

oscillatory signal over 7 min to produce a value for average basal blood flow. This is fairly simple to perform and is indeed the method normally used to express laser Doppler flowmetry data. There is in addition, however, interesting and potentially important information in the oscillatory pattern seen in the laser Doppler flowmetry output prior to contralateral hand warming, but unfortunately it has so far proved impossible to express this output quantitatively. This is due principally to its irregularity so that there is no readily available mathematical or computational programme which can adequately describe this biological phenomenon.

Prazosin is a specific α_1-adrenoceptor antagonist. As such, it could theoretically have two different effects on skin blood flow. If it reduced systemic blood pressure, then the skin blood flow could be reduced purely due to a fall in the upstream perfusion pressure. On the other hand, prazosin could increase skin microcirculation due to its peripheral vasodilatory effect. In this particular study we can be fairly confident for several reasons that all of the effects of prazosin are due to effects on the skin microcirculation itself rather than due to systemic haemodynamic effects.

Firstly, we deliberately choose a low dose of prazosin so that, there were no changes in systemic blood pressure, heart rate or, as has been shown previously, plasma noradrenaline (Elliott *et al.*, 1981; Bateman *et al.*, 1979). Secondly, there were also no BP or HR changes during the cold immersion test and this suggests that the effects of prazosin were limited to the microcirculation not only at rest but also during the cold immersion test. Thirdly, Mancia *et al.* (1980) have shown that at even higher doses of prazosin there are still no effects on the systemic haemodynamic response to cold immersion test.

It has previously been shown from direct invasive measurements that there is increased skin sympathetic activity in response to both deep inspiration and cold immersion (Hagbarth *et al.*, 1972; Delius *et al.*, 1972). This is the first time that laser Doppler flowmetry has been used to study these phenomena, and more importantly, this is the first time that such phenomena have been quantified. The inhibitory effect of prazosin on these two reflexes clearly establishes that postsynaptic α_1-adrenoceptors are involved in these reflexes.

Since presynaptic receptors are of the α_2-subtype, changes in presynaptic function or NA release are unlikely to contribute to the observed effect of prazosin. Postsynaptic receptors are of course, a mixture of α_1,- α_2- and β-adrenoceptors (Flavahan & Vanhoutte, 1986). This is perhaps part of the reason why prazosin only attenuated rather than completely blocked these reflexes.

We have found that prazosin caused an increase in resting skin blood flow 2 h after its ingestion. This suggests that in the normal basal state, the SNS does maintain some vasoconstrictor tone. It is also interesting that the prazosin induced increase in resting skin blood flow is no longer evident after contralateral hand warming. This supports a previous hypothesis (Bini *et al.*, 1980) that hand vasodilatation is merely due to the release of vasoconstrictor tone rather than an active process *per se*.

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