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# A pragmatic approach to the pressor dose-response as an index of vascular reactivity and adrenoceptor function in man

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**1** In clinical pharmacological studies, where it is not possible to describe the full dose-response curve, the construction of dose-response relationships ideally depends upon achieving a reproducible and readily measurable response for each dose administered.
**2** This study investigates in normotensive males the technique of dose-response analysis for the blood pressure and heart rate increases with constant infusions of incremental doses of vasoactive drugs, particularly catecholamines.
**3** Steady state responses could be adequately obtained using 5 min infusion periods (at each dose level) for noradrenaline and α-methyl noradrenaline. At least 7 min was required for phenylephrine and 8 min for isoprenaline.
**4** There was an approximate correlation between the time to achieve the steady state response and the half-life of the offset of the agonist effect.
**5** For interindividual comparisons it is desirable to compare steady state responses and so the time at each dose level will vary according to which agonist is being used.
**6** For intraindividual comparisons it may not be essential that steady state responses are achieved. For example, assessment of the effect of prazosin on the responses to phenylephrine, by calculation of dose ratios, indicated that 5 min dose intervals were adequate.

**Keywords** dose-response vascular reactivity adrenoceptor function

## Introduction

In experimental pharmacology the haemodynamic responses to increasing doses of vasoactive agonists and hormones are widely used to test α- and β-adrenoceptor function and vascular responsiveness. However, because it is not possible to achieve maximum haemodynamic responses in man there is a lack of detailed information about the optimal technique to be employed for each agonist in clinical studies *in vivo*. Typically the agonist is administered in stepwise incremental doses, either as a constant intravenous infusion for determination of 'steady state' responses, or as intravenous bolus doses for measurement of peak responses. The major practical disadvantage of the bolus method is the difficulty of accurately recording the peak response without using a direct i.e. invasive means of blood pressure measurement. Indirect blood pressure measurement is suitable for the infusion approach but the optimal infusion technique has not been established. In particular the time required for each agonist or hormone to produce a steady state response has not been well defined. The present study was designed to investigate the time course of pressor responses in man and thereby to attempt to calculate the optimal infusion times for a series of agonists which are commonly used in human pharmacological studies.

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## Methods

The study population comprised nine young healthy male normotensives, 20–38 years, with each individual agonist study being performed on a cohort of six of these subjects in a randomised, single-blind manner. The agonists examined were noradrenaline, α-methyl noradrenaline, phenylephrine, angiotensin II, adrenaline and isoprenaline and for each agonist at least three and up to six different dose levels were administered (Table 1). The phenylephrine infusion protocol was also undertaken on a separate occasion 2 h after the oral administration of 1 mg prazosin, a selective $\alpha_1$-adrenoceptor antagonist.

Subjects attended the Clinical Pharmacology Research Unit having abstained from alcohol, nicotine and caffeine from 22.00 h on the previous evening. They remained supine throughout the study period. After the insertion of an intravenous cannula into each arm and after an initial period of at least 30 min of supine rest an intravenous infusion was commenced into a forearm vein via an infusion pump (Braun Perfusor). For the first infusion period of 20 min 0.9% normal saline solution was administered and thereafter each of a series of incremental doses of agonist was infused, each dose level for 20 min. Finally, the infusion of active drug was terminated and the subjects were observed for a further 20 min period or longer until blood pressure and heart rate returned to pre-infusion levels. The total volume of fluid infused was 200–300 ml. The total duration of the study was therefore between 100 and 160 min and during this time minute-by-minute recordings of heart rate and blood pressure were measured by a semi-automated sphygmomanometer (Sentron) and ECG recording from standard praecordial leads. Targets of 45 mm Hg systolic and 30 mm Hg diastolic blood presure, and 60 beats $min^{-1}$ for heart rate, were set for the increases in blood pressure and heart rate respectively. When any one of these targets was reached the incremental infusion was stopped.

**Table 1** Drugs and hormones studied and dose ranges used

| | *Dose range* ($\mu g\ kg^{-1}\ min^{-1}$) |
|---|---|
| Noradrenaline | 0.02–0.5 |
| α-methyl noradrenaline | 0.02–1.0 |
| Adrenaline | 0.02–0.5 |
| Phenylephrine (alone) | 0.5–5.0 |
| Phenylephrine (after prazosin) | 0.5–10.0 |
| Isoprenaline | 0.01–0.05 |
| Angiotensin II | 1.0–20 |

For each infusion period of 20 min, blood pressure and heart rate data were grouped into four intervals, each of 5 min (i.e. 1–5, 6–10, 11–15 and 16–20 min). The readings in each of these four intervals were averaged; the average baseline (i.e. pre-infusion) reading was then subtracted, and the four sets of averages were plotted against the logarithm of the rate of infusion.

All results were fitted to a model of the form

$$\Delta BP = A.I^2 + B.I + C$$

where $\Delta BP$ = change in blood pressure
and I = log (rate of infusion).

This method has been described in detail elsewhere (Sumner *et al.*, 1982). The dose rate of agonist required to raise the blood pressure by 10, 15 or 20 mm Hg (or the heart rate by 20 beats $min^{-1}$ in the case of isoprenaline) was calculated. These dose rates were designated $PD_{10}$, $PD_{15}$, $PD_{20}$ and $CD_{20}$ respectively.

For the experiment involving pretreatment with prazosin comparison of the dose-response curve involved calculation of the dose ratio, which is defined as

$$\text{Dose ratio} = \frac{PD_{15} \text{ with prazosin}}{PD_{15} \text{ without prazosin}}$$

The fall in blood pressure after the active infusion was stopped was fitted to a function of the form

$$P = P_0 + P_1\, e^{-\alpha t}$$

where $P$ = Pressure at time $t$
$P_0$ = Final pressure (as $t \rightarrow \infty$)
$P_0 + P_1$ = Pressure at $t = 0$
$\alpha$ = Decay constant
(Note that half-life of decay $= \frac{0.693}{\alpha}$).

Results throughout are expressed as mean ± s.d. and the statistical evaluation of the values calculated for the different time intervals was by Student's *t*-test, with Bonferroni's correction to allow for multiple comparisons.

## Results

The results for all agonists are summarised in Table 2 and in Figures 1, 2 and 3.

In the figures the average $PD_{20}$, $CD_{20}$ etc. obtained for the various 5 min intervals, is expressed as a percentage of the presumed steady state value i.e. the value obtained for the 16–20 min interval. For all agonists, although the scatter of data was large, the values obtained for the

**Table 2** Dose-responses

| | *Agonist* | *Time period (min)* 1–5 | 6–10 | 11–15 | 16–20 |
|---|---|---|---|---|---|
| 1 | Noradrenaline (systolic $PD_{20}$) | 0.14 ± 0.13 | 0.12 ± 0.08 | 0.13 ± 0.08 | 0.12 ± 0.08 |
| 2a | Phenylephrine (systolic $PD_{15}$) | 2.0 ± 1.6 | 1.4 ± 1.2 | 1.4 ± 1.0 | 1.3 ± 0.9 |
| 2b | Phenylephrine (after prazosin) (systolic $PD_{15}$) | Insufficient data | 3.7 ± 1.5 | 3.6 ± 1.6 | 2.7 ± 1.1 |
| 3 | α-methyl noradrenaline (systolic $PD_{20}$) | 0.56 ± 0.30 | 0.55 ± 0.27 | 0.48 ± 0.24 | 0.50 ± 0.33 |
| 4 | Angiotensin II (diastolic $PD_{20}$) | 14.6 ± 7.3 | 11.2 ± 4.7 | 12.1 ± 4.7 | 11.9 ± 7.2 |
| 5 | Adrenaline (systolic $PD_{10}$) | 0.10 ± 0.05 | 0.09 ± 0.09 | 0.08 ± 0.07 | 0.09 ± 0.07 |
| 6 | Isoprenaline ($CD_{20}$) | 0.022 ± 0.006 | 0.017 ± 0.005 | 0.016 ± 0.005 | 0.015 ± 0.005 |

11–15 min interval were not significantly different from those for the 16–20 min interval, consistent with the attainment of a steady state response.

*Noradrenaline*

The maximum rise in systolic pressure was 46 ± 14 mm Hg and in diastolic pressure 33 ± 12 mm Hg. The maximum change in heart rate was −14 ± 5 beats $min^{-1}$. For the purposes of this analysis, because of the larger rise obtained in systolic pressure, $PD_{20}$s have been calculated from systolic pressures only. There was no statistically significant difference between the $PD_{20}$s obtained from any of the 5 min intervals; although it should be noted in Figure 1 that there is up to a 20% difference between the $PD_{20}$ calculated from the 16–20 min values and that calculated from earlier times.

For the decay phase, the half life for the progressive fall in systolic pressure was invariably less than 3 min in all individual subjects.

*Phenylephrine*

The maximum rise in systolic pressure ws 47 ± 15 mm Hg, and in diastolic pressure 38 ± 12 mm Hg. The maximum change in heart rate was −24 ± 7 mm Hg. To maintain consistency and allow direct comparison with other agonists, systolic pressure was again used. In this study a rise of 20 mm Hg was not obtained in every subject and so the $PD_{15}$ was used for comparative analysis. There was no significant difference between the $PD_{15}$ values obtained using the 6–10, 11–15, or 16–20 min intervals; however, the $PD_{15}$ values obtained from the 1–5 min interval were significantly higher than the other intervals; this discrepancy can be as high as 50% (see Figure 1). This indicates that the pressure rise achieved after 5 min infusion is substantially less than the pressure rise achieved after 20 min, i.e. steady state had certainly not been achieved within the first 5 min of the infusion.

For the decay phase, the fall in mean arterial pressure gave a mean half-life of about 4 min (95% confidence interval [2.6, 5.3] min).

In the presence of prazosin the dose-response curves for phenylephrine were significantly shifted to the right. The $PD_{15}$ values obtained for the 1–5 min interval were again significantly higher than those obtained for later intervals. However, the dose ratio for the 1–5 min interval was not significantly different from the dose ratios calculated for later intervals. Thus, for this comparative circumstance the 5 min infusion period was adequate.

*α-methyl noradrenaline*

The maximum rise in systolic pressure was 45 ± 19 mm Hg but the increase in diastolic pressure was only 10 ± 15 mm Hg. The maximum change in heart rate was 5 ± 11 beats $min^{-1}$. The $PD_{20}$s were calculated only from systolic pressures. The results were essentially similar to those obtained with noradrenaline. A steady state

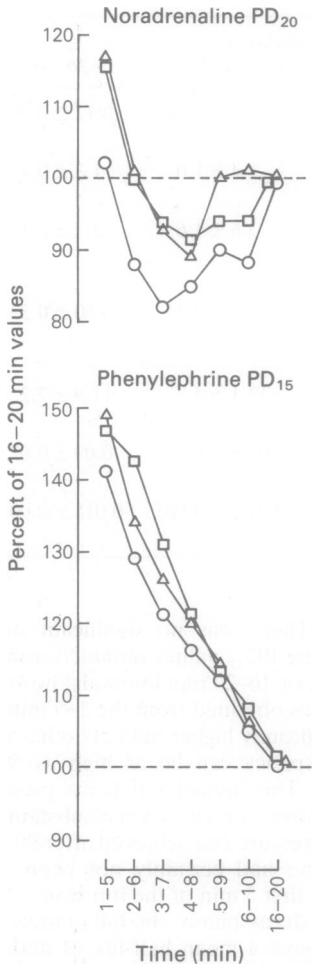


**Figure 1** Averages of each 5 min period expressed as a percentage of the final 5 min period for the increases in systolic (□), diastolic (△) and mean (○) arterial pressure due to noradrenaline and phenylephrine. The typical s.d. on each data point was respectively ±20 and ±30.

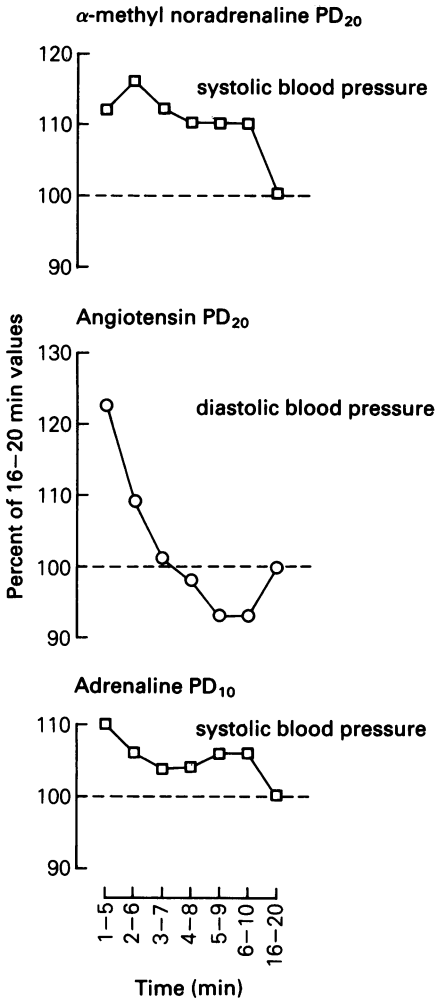


**Figure 2** Averages of each 5 min period expressed as a percentage of the final 5 min period for the pressor responses to α-methyl noradrenaline, angiotensin II and adrenaline. The typical s.d. on each data point was respectively ±20, ±20, ±25.

response had been reached within 5 min of infusion and the half-life of offset was less than 3 min.

*Angiotensin II*

The maximum rise in systolic pressure was 30 ± 15 mm Hg and in diastolic pressure was 33 ± 3 mm Hg. The maximum change in heart rate was −9 ± 12 beats $min^{-1}$. Although the average maximum rises in systolic and diastolic pressure were comparable, the between-subject variability in systolic pressure was much greater than that in diastolic pressure; $PD_{20}$s were therefore calculated from diastolic pressures only. There was no significant difference between the $PD_{20}$s obtained from any of the 5 min intervals, although again it should be noted that the mean $PD_{20}$ obtained (see Figure 1) ranged between 93% and 123% of the 16–20 min value.

*Adrenaline*

The maximum rise in systolic pressure was 34 ± 14 mm Hg and in diastolic pressure 7 ± 8 mm Hg. The maximum change in heart rate was 13 ±

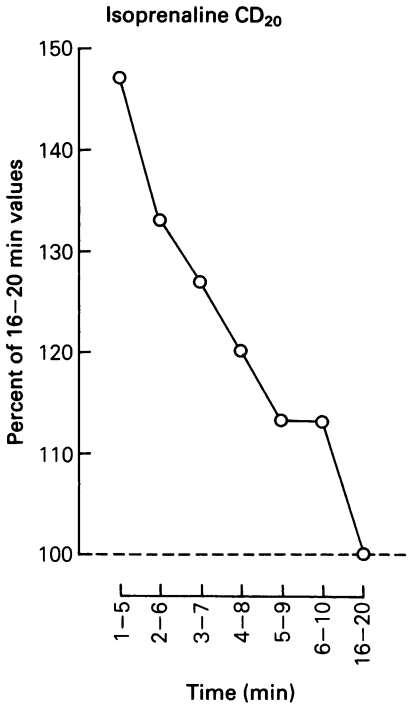


**Figure 3** Averages of each 5 min period expressed as a percentage of the final 5 min period for the chronotropic response to isoprenaline. The typical s.d. on each data point was ±35.

12 beats $min^{-1}$. $PD_{10}$s were calculated from systolic pressures. There was no significant difference between the $PD_{10}$s obtained from any of the 5 min intervals. The half-life of offset of both the blood pressure and the heart rate changes was always less than 3 min.

*Isoprenaline*

The average maximum rise in heart rate observed was 63 ± 8 beats $min^{-1}$. The maximum change in systolic blood pressure was 19 ± 7 mm Hg and in diastolic blood pressure −11 ± 6 mm Hg. There was no significant difference between the $CD_{20}$ values for 6–10, 11–15, or 16–20 min intervals but the $CD_{20}$ values obtained for 1–5 min were significantly higher than the other intervals. By a similar argument to that used with phenylephrine, this indicates that a steady state response has certainly not been achieved after 5 min of infusion. Figure 3 shows the size of the error involved in calculating the $CD_{20}$ after different infusion times. The half-life of the recovery of heart rate ranged between 3–16 min, with a mean value of 7 min.

## Discussion

Although pressor response relationships following agonist infusions are widely used in the clinical investigation of α- and β-adrenoceptor antagonist drugs, little attention appears to have been paid to the optimal infusion technique. This is particularly important as maximum responses are not possible in human experiments and only the lower part of the dose-response relationship can be explored. Thus, misleading results can be obtained if the period for which each dose is administered is less than the time required to reach a steady-state response. In general, this error will overestimate the $PD_{20}$, with the resultant possible interpretation of a relatively reduced responsiveness.

For example, for angiotensin II, the $PD_{20}$ obtained from the 1–5 min interval is 23% higher than the steady state value; if we take the 2–6 min value the difference is only 9%. In comparison with the other agonists, the difference observed is choosing the 1–5 min interval is substantially higher for phenylephrine and isoprenaline. For phenylephrine the $PD_{15}$ (systolic) using 1–5 min is 47% higher than the steady state; for 6–10 min it is 8% higher. Corresponding figures for isoprenaline are 47% and 13%. For the pressor response to phenylephrine the errors are very similar whether systolic, diastolic or mean pressure is used.

In practical terms, for the α-adrenoceptor agonists noradrenaline and α-methyl noradrenaline and for angiotensin II, a 5 min infusion period, at each dose level, appeared adequate for achieving a steady state response, as judged by the absence of significant differences between the $PD_{20}$ values obtained during different time intervals. However, for isoprenaline and phenylephrine, 5 min was not sufficient. For these two agonists an infusion time of at least 10 min would be desirable. However, practical problems might arise because of the total time required for a study involving four or more dose levels and in most cases 7 min infusion periods gave adequate results. Although these studies were undertaken in healthy normotensive volunteers, there is no *a priori* evidence to suggest that a different pragmatic approach will be required for hypertensive patients, even although the magnitude of the response or the doses required may be different. The large scatter of the data presents a more immediate problem, however, with varying degrees of intra-individual variability for each agonist. To some extent the power of this type of pressor dose-response study depends on the selectivity of the agonist used. For example, with phenylephrine the intra-

subject variability is very much less than the inter-subject variability, such that the power to detect a 20% change in the $PD_{15}$ is greater than 95%, even with only six subjects. With α-methyl-noradrenaline the corresponding power is about 50% and with noradrenaline it is less than 50%.

It might be expected that the time to reach the steady state response (i.e. the increase in blood pressure or heart rate) would be correlated with the rate at which the blood pressure or heart rate returned to normal after cessation of the agonist infusion. An additional approach was therefore to investigate the offset or decay phase of the blood pressure response using a single exponential function. If the kinetics of blood pressure response to the agonist are linear then the blood pressure rise during a constant infusion would be 75% of its steady state value after two offset half-lives, 88% after three half-lives and 94% after four half-lives. In fact the half-lives calculated during the offset phases were approximately correlated with the time to steady state response. Thus for noradrenaline, adrenaline, α-methyl-noradrenaline and angiotensin with offset half-lives in general less than 3 min, infusion periods of 5 min were adequate. For isoprenaline and phenylephrine, with offset half-lives significantly longer than 3 min, infusion periods of more than 5 min are required. In our study we did not discern any significant difference in the offset time for heart rate compared with blood pressure, following adrenaline, and the rate of offset was also comparable to that seen with noradrenaline and isoprenaline. This is slightly at variance with other reports (Brown & Dollery, 1984; Fellows *et al.*, 1985) which have shown that heart rate changes persisted for at least 30 min after cessation of adrenaline. However, a significant post-infusion effect has not been a consistent finding with adrenaline and the observed differences may simply reflect the dosage and total duration of the infusion.

In practice, in clinical pharmacology, it is often not the absolute value for $PD_{20}$ that is of most interest, but the relative values under different conditions; typically the dose ratio of the $PD_{20}$ measured in the presence of an antagonist drug to the $PD_{20}$ measured under placebo conditions. It is apparent that such a ratio may not be as dependent upon the time of infusion as the $PD_{20}$ itself, since the $PD_{20}$s under both sets of circumstances may be overestimated by approximately the same proportion if the time is less than that required for steady state on both occasions. The results with the $\alpha_1$-adrenoceptor agonist phenylephrine with and without the α-adrenoceptor antagonist prazosin lend qualified support to this view, in so far as there were no significant differences in dose ratios between any of the 5 min intervals. However, the variation between times is large and a bigger sample would be required to form a definite conclusion.

In summary, dose-response curves in clinical pharmacological studies may be satisfactorily characterised using incremental dosage steps of 5–10 min. The exact time required for the infusion of a particular agonist depends on what magnitude of error the experimenter deems acceptable and, as already discussed, if the requirement is for a dose-response within 20% of the steady state value, an infusion of 5 min is adequate for the agonists noradrenaline, α-methyl-noradrenaline, and adrenaline; whereas at least 6 min is required for angiotensin II, 7 min for phenylephrine and 8 min for isoprenaline. For the usual circumstance of comparative analysis, however, it may be possible to achieve the same erorr by shorter infusion times.

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