The pressor dose-response in clinical cardiovascular pharmacology

D. J. SUMNER¹ & H. L. ELLIOTT

¹Department of Clinical Physics and Bioengineering West of Scotland Health Boards and University Department of Materia Medica, Stobhill General Hospital, Glasgow G21 3UW

Introduction

The controlled administration of increasing doses of agonist drugs is widely used in general pharmacology to define dose-response relationships and thereby investigate the responses mediated via specific receptors. In clinical cardiovascular pharmacology, this approach can be applied to studies of peripheral blood vessels or the heart, and the function of the autonomic nervous system. For example, overall vascular responsiveness mediated via α -adrenoceptors can be assessed by incremental intravenous infusions of pressor amines such as noradrenaline, α -methyl noradrenaline and phenylephrine and similarly infusions of isoprenaline, or other β -adrenoceptor agonists, can be used to investigate the responsiveness of both vascular and cardiac β -adrenoceptors.

In most circumstances the procedure is based upon the intravenous administration of the chosen agonist through a series of different dose rates, with measurement of heart rate and blood pressure when a reproducible response, either steady-state or peak response, has been reached at each dose level. In general, a line or curve is then drawn through the set of data points which represents the responses at each particular dose of agonist; for most of the agonists mentioned above this response will usually be blood pressure but for isoprenaline it will often be heart rate. While it may be possible to measure the maximal heart rate responses to individual bolus doses of isoprenaline it is usually necessary with non-invasive blood pressure recording to measure the 'steady state' response to an infusion. The major limitation in human studies is that, because it is not possible to determine maximum effect, interpretation of the dose-response relationship relies on the attainment of an arbitrarily predetermined response; for example, the dose of agonist which produces a blood pressure increase of 20 mm Hg, or a heart rate increase of 20 beats

min⁻¹. The exact value for this dose is often obtained by interpolation; the doses for 'pressor' responses of 20 mm Hg or 'chronotropic' responses of 20 beats min⁻¹ may be represented by PD₂₀ and CD₂₀ respectively.

It is frequently of interest to assess responsiveness in an individual subject under different circumstances, usually in the presence of a receptor antagonist drug. In general an antagonist drug will cause a rightwards shift of the dose-response curve such that larger doses of agonist will be required to produce responses comparable to those obtained when the antagonist drug was not present. The effect of the antagonist can be conveniently assessed by the *dose ratio*, which is defined as the ratio of the PD₂₀ when the antagonist is present to the PD₂₀ when no antagonist is present. The dose ratio is particularly useful in clinical studies because of the practical and ethical limitations to the extent to which the dose-response relationship can be defined. Because it is not generally possible to explore the full dose-response curve (which often has a sigmoid configuration) and hence measure the D₅₀ (i.e. the dose required to obtain 50% of the maximum response) comparison of the full dose-response curves is not readily possible in human studies. Nevertheless, as in classical pharmacological experiments, for a competitive antagonist the dose ratio should be *constant* irrespective of the particular increase in blood pressure that is selected (Arunlakshana & Schild, 1959), reflecting the parallel shift of the dose-response curve. In contrast, with a non-competitive antagonist the dose ratio will not be constant but will vary according to the response level chosen for the comparison. This obviously reflects the lack of a parallel shift.

We will now consider the practical techniques of agonist dose-response studies, and some of the problems involved in their analysis.

General considerations

The safety of the individual subject is of paramount importance and studies should be undertaken under adequate clinical supervision (in our research unit, the physician and the research nurse) in a quiet, temperature-controlled environment. Subjects should remain supine throughout the whole infusion period. An intravenous cannula is inserted into a forearm vein and after a period of not less than 30 min further recumbent rest an intravenous infusion is started using a suitable infusion pump such as the Braun Perfusor series. The essential requirements of the infusion pump are precision with small fluid volumes, a reasonable range of delivery rates and smooth 'gearing' to permit immediate rate changes. In our Research Unit we are able to deliver accurately $250 \mu\text{l min}^{-1}$ through to 5 ml min^{-1} via the Braun Perfusor E pump.

Initially 0.9% saline is infused (volume approximately 2.5 ml) while the baseline recordings of heart rate and blood pressure are obtained and then the agonist infusion is commenced. The dose ranges of the commonly used agonists are shown in Table 1 and the mean doses required to produce arbitrarily pre-determined effects are also shown. Repeated, minute-by-minute haemodynamic measurements are recorded throughout the whole infusion period using a semi-automated sphygmomanometer (e.g. Sentron, Arteriosonde, Datascope) and an ECG obtained from standard praecordial leads. In pressor response studies in young normotensives our aim is to produce a controlled increase of 20–25 mm Hg in mean arterial pressure but, in the interests of safety, upper safety limits of 45 mm Hg systolic and 30 mm Hg diastolic are not exceeded. If these limits are reached then the infusion pump is immediately switched off. Similarly, for chronotropic studies in healthy young subjects the target increment is 45 beats min^{-1} , with an upper limit of 60 beats min^{-1} ,

or an absolute heart rate of greater than 140 beats min^{-1} .

Time to achieve steady state responses

The time to produce 'steady state' responses in man has not been well defined for all agonists. We have recently completed a study (Sumner *et al.*, 1987) designed to investigate the time course of pressor responses and thereby to calculate the optimal infusion times for a series of agonists. Not surprisingly, the exact time required for the infusion of a particular agonist depends on what magnitude of error in the PD_{20} the experimenter deems acceptable; if the requirement is for a value of the PD_{20} 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.

As noted previously, it is often not the absolute value of PD_{20} that is of most interest, but the dose ratio. Such a ratio may not be as dependent upon the time of infusion as the value of the PD_{20} itself, even if the infusion period is less than that required for steady state, since the PD_{20} under both sets of circumstances may be overestimated by approximately the same proportion on both occasions. Results with the α_1 -adrenoceptor agonist phenylephrine with and without the α_1 -adrenoceptor antagonist prazosin lend qualified support to this view (Sumner *et al.*, 1987).

Method of analysis

It is not clear what is the most appropriate method of analysing pressor dose-response studies when the data may comprise only four or five points, each subject to error, and when the functional relationship between dose and effect

Table 1 Dose ranges and typical mean dose rates required in human dose-related studies

Agonist	Dose range ($\mu\text{g kg}^{-1} \text{ min}^{-1}$)	Mean dose (response) $\pm$ s.d.
Phenylephrine	0.5–5.0	1.5 ± 1.2 (PD_{15})
α -methyl noradrenaline	0.01–1.0	0.52 ± 0.29 (PD_{20})
Noradrenaline	0.02–0.5	0.13 ± 0.10 (PD_{20})
Adrenaline	0.02–0.5	0.10 ± 0.07 (PD_{10})
Isoprenaline	0.005–0.05	0.019 ± 0.005 (CD_{20})
Angiotensin II	1.0–20 ($\text{ng kg}^{-1} \text{ min}^{-1}$)	11.7 ± 5.5 (PD_{20})

is not readily apparent. The equation most commonly used to fit complete pressor dose-response curves can be written in the form:

$$E = \frac{E_{\max} \left(\frac{D}{D_{50}} \right)^n}{1 + \left(\frac{D}{D_{50}} \right)^n} \quad (1)$$

where $E_{\max}$ = Maximum response
 D_{50} = Dose at which 50% response is produced.
 n = Number of agonist molecules combining simultaneously.

If E is plotted against D a curve of the form shown in Figure 1a is obtained; if E is plotted against $\log D$ a sigmoid curve is obtained (Figure 1b).

Dose-response curves have traditionally been represented as in Figure 1b, since the relationship is linear over a considerable range of doses. However, this advantage does not necessarily apply to clinical dose-response studies, where only the lower part of the curve can be obtained, and only a fraction of the linear portion will be accessible; as we shall see, other transformations may be more appropriate.

Because $E_{\max}$ is never known in clinical studies it is not possible to fit equation (1) to a set of pressor response data. The investigator has essentially a choice between fitting the data to a

model which provides an empirical description of the drug effects, or using mechanistic models derived from equation (1) (Endrenyi, personal communication).

Sumner *et al.* (1982) showed that in most cases a quadratic function was an appropriate function to fit the lower curved portion of a log-dose response curve; this is clearly an example of an empirical model. For practical purposes, however, it would be more convenient if a *linear* fit of some kind could be used. The methods usually employed for linearising equation (1) require a knowledge of $E_{\max}$; however, there are two possible methods that can be used where $E_{\max}$ is not known, both of which could be categorised as 'mechanistic' models.

(1) For values of $D \ll D_{50}$, the relationship between E and D is often approximately linear. In this case a non-specific association can be considered, or perhaps a specific one of the Michaelis-Menten type with all doses being lower than the Michaelis constant (Endrenyi, personal communication).

(2) When $D \ll D_{50}$, $E \approx E_{\max} \left(\frac{D}{D_{50}} \right)^n$

Taking logarithms of both sides,

$$\log E = \log \left(\frac{E_{\max}}{D_{50}^n} \right) + n \log D$$

so that, if the logarithm of change in blood pressure is plotted against the logarithm of dose, a straight line should be obtained, with a slope of

Figure 1 (a) response plotted against D , (b) response plotted against $\log D$.

n. The slope measures the extent of deviations from Michaelis-Menten type of behaviour.

We have examined pressor response curves from eight different studies, and fitted them to all three of the above models. Examination of residual and normal probability plots indicates a relatively low proportion of acceptable fits, although the quadratic model was superior to linear model (1) which in turn was superior to linear model (2). This is not altogether surprising, since the quadratic model contains an additional parameter.

Sumner *et al.* (1982) pointed out that if there is prior evidence that the antagonist is a competitive one, the pressor responses obtained with antagonist and placebo should be fitted simultaneously to a reduced model of a linear or quadratic function and a parallel shift between the two curves. It is important, therefore, to test the performance of various models not just in their ability to fit individual dose-response curves, but also in the simultaneous fitting of curves with and without antagonist present. Preliminary results on our data indicate that, as in the individual fits, the quadratic model is usually superior to the linear models, although both antagonist and agonist influence which method of fitting is most appropriate.

A modification of the simultaneous fitting procedure is to allow a vertical as well as horizontal shift of the curves (Jamieson, personal communication). This could be justified on the grounds that the baseline pressure or heart rate was in error. Not surprisingly, the fit is better, but the values obtained for the dose ratio may be quite different from those obtained with a horizontal shift model. It is likely that reproducibility studies (see below) will be required to discriminate between these models.

Simulations

The adequacy or otherwise of a particular model in fitting a set of dose-response curves does not in itself give us any information as to whether that model produces an accurate and unbiased estimate of the dose ratio. We have undertaken a series of studies using simulated dose-response curves with a predetermined dose ratio, and fitted part of these curves to the three models described above. Results (Howie, Ph.D. thesis, in preparation) show that the estimate of dose ratio is very sensitive to the proportion of the dose-response curve observed, the number of doses and intervals between doses. There is no one model to be generally preferred. Conse-

quently, it would be advisable to fit a set of pressor response data to all three models, using simultaneous fits where appropriate, and use results from the model that seems the most satisfactory for that particular set of data.

Reproducibility and sources of variation in pressor dose-response studies

Little information is available on the reproducibility of pressor dose-response studies in man and factors influencing their variability. There is some evidence of a circadian variation in α - and β -adrenoceptor responsiveness, at least in rats (Wirz-Justice *et al.*, 1980), so it is conceivable that the PD₂₀ might show similar variations. In a study of the concentration-effect relationships for the α_1 -adrenoceptor antagonist trimazosin we found variations in the PD₂₀ for phenylephrine administered under placebo conditions, with values higher in the afternoon than in the morning. Whether or not this represents a genuine circadian rhythm, or is a reflection of other haemodynamic, hormonal, or biochemical changes, remains unclear at present and is the subject of current investigation.

There is evidence that physical conditioning increases the effect of circulating adrenaline and noradrenaline on systolic blood pressure (Le Blanc *et al.*, 1977; Mann *et al.*, 1984). The effect of hypertension on the pressor response is not clearly defined; the situation is complex, as the baseline pressure is raised and there are alterations in baroreflex mechanisms and the geometry of resistance vessels. Goldenberg *et al.* (1948) have shown that hypertensive patients are more sensitive to noradrenaline than normotensive subjects but this is not a universal finding and conflicting results exist (Collste & Ekeröth, 1986).

Elliott *et al.* (1982) showed in a group of elderly patients that the PD₂₀ for phenylephrine infusions was greater than the PD₂₀ in young volunteers, suggesting a reduced sensitivity in the elderly, although this may be partly attributable to the slightly higher baseline blood pressures.

Inter-subject variability in response will obviously be dependent on pharmacokinetic differences for the administered agonist. There is only limited information about concentration-effect relationships in man for noradrenaline and phenylephrine (Collste & Ekeröth, 1986; Martinsson *et al.*, 1986) and to date no clear concentration-effect relationships have been defined.

Conclusions

Evaluation of peripheral vascular pressor responsiveness and other agonist-induced haemodynamic responses provides a useful technique in cardiovascular clinical pharmacology. However, there are a number of problems in the execution and analysis of human studies, particularly if patho-physiological aspects are being investigated. For example, the 'ideal' approach should measure a reproducible response and this requires the attainment of a steady state response at each dose level, with infusion of the agonist for a time sufficient to produce this. As discussed, the required steady state time for each agonist has not been well defined and in most circumstances it seems that the pragmatic use of 7 or 8 min at each dose level will provide satisfactory results. Our limited experience suggests that reasonably reliable values for dose ratios may be obtained without achieving steady state responses; however, this approach must be used

with caution and is clearly not as satisfactory as obtaining pressor dose-responses in which a steady state is achieved at each dose level.

The individual response to pressor amines seems to be a very variable quantity and may depend on environmental conditions as well as time of day and the haemodynamic status of the individual. Further clarification of the relative importance of these factors is awaited, but in the meantime it is essential at least to minimise extraneous sources of variability by standardising the laboratory conditions, equipment and experimental design.

In the analysis all data points from each individual should be included; different mathematical models (linear, quadratic) should be tested and compared, and the most appropriate one selected. Finally, it is important to carry out simultaneous fitting when competitive antagonists are used, as a single estimate of dose ratio, which is independent of response, can be obtained.

References

- Arunlakshana, O. & Schild, H. O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac.*, **14**, 48–58.
- Collste, P. & Ekeröth, M. (1986). Noradrenaline infusion in hypertensive patients and healthy volunteers. *3rd World Conference on Clinical Pharmacology & Therapeutics, Stockholm*.
- Elliott, H. L., Sumner, D. J., McLean, K., Rubin, P. C. & Reid, J. L. (1982). Effect of age on vascular alpha-adrenoceptor responsiveness in man. *Clin. Sci.*, **63**, 305–308.
- Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G. & Roh, C. E. (1948). The haemodynamic response of man to nor-epinephrine and epinephrine and its relation to the problem of hypertension. *Am. J. Med.*, **5**, 792–806.
- Le Blanc, J., Bronlay, M., Dulac, S., Jobin, M., Labrie, A. & Rousseau-Migneron, S. (1977). Metabolic and cardiovascular responses to norepinephrine in trained and nontrained human subjects. *J. appl. Physiol.*, **42**, 166–173.
- Mann, S. J., Krakoff, L. R., Fallon, K. & Yeager, K. (1984). Cardiovascular responses to infused epinephrine: effect of the state of physical conditioning. *J. cardiovasc. Pharmac.*, **6**, 339–343.
- Martinsson, A., Bevegård, S. & Hjemdahl, P. (1986). Analysis of phenylephrine in plasma: Initial data about the concentration-effect relationship. *Eur. J. clin. Pharmac.*, **30**, 427–431.
- Sumner, D. J., Elliott, H. L. & Reid, J. L. (1982). Analysis of the pressor dose response. *Clin. Pharmac. Ther.*, **32**, 450–458.
- Sumner, D. J., Elliott, H. L., Vincent, J. & Reid, J. L. (1987). A pragmatic approach to the pressor dose-response as an index of vascular reactivity and adrenoceptor function in man. *Br. J. clin. Pharmac.*, **23**, 505–510.
- Vincent, J., Elliott, H. L., Meredith, P. A. & Reid, J. L. (1985). Trimazosin: Relationships between hypotensive effect, vascular responsiveness and drug and metabolite concentrations. *J. cardiovasc. Pharmac.*, **7**, 913–918.
- Wirz-Justice, A., Kafka, M. S., Naber, D. & Wehr, T. A. (1980). Circadian rhythms in rat brain alpha- and beta-adrenergic receptors are modified by chronic imipramine. *Life Sci.*, **27**, 341–347.

(Received 3 September 1986,
accepted 12 January 1987)