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**THE ASSESSMENT OF
 β -ADRENOCEPTOR BLOCKING DRUGS IN MAN**

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Within the last 10–15 years, the medical profession began to manifest a new interest in the properties which drugs exhibited in man. Prior to this, pharmacology had been primarily directed at studies in animals, and it was largely assumed that drugs would not behave distinctly differently in man. What occasioned the change is not clear—it may have been the development of increasingly sophisticated methods for measurement of physiological parameters in man, or, possibly an awareness, following the thalidomide disaster, that doctors did not have as much basic information about the drugs which they habitually used as they ought to. Thus the discipline of clinical pharmacology began to emerge both in Europe and in North America.

Interest focused first on drugs which produced easily measurable effects both in normal subjects and in patients and this inevitably meant that drugs acting on the cardiovascular system came under the closest scrutiny. This situation coincided with the introduction of a new class of compound, β -adrenoceptor antagonists, which were of immense interest both to physiologists and pharmacologists, were available for administration to man and which, on theoretical grounds, were expected to influence heart rate, cardiac contractility and blood pressure. The result has been that in a relatively short period of time, β -adrenoceptor blocking drugs have been widely investigated and the story of their investigation is, in many ways, a history of the advances and limitations of clinical pharmacology to the present time. This review examines some aspects of the assessment of β -adrenoceptor blocking drugs in man.

Ahlquist (1948) classified adrenoceptors into two groups, based on the effects of six sympathomimetic amines on a variety of effector systems. He suggested that there were two types of adrenoceptor which he designated α and β . The distribution of these receptors is shown in Table 1, which also shows the responses produced by activation of these receptors. β -adrenoceptors have subsequently been further divided into two main groups, β_1 receptors in the heart and β_2

receptors in the bronchi and blood vessels (Lands, Luduena & Buzzo, 1967). With the discovery of dichloroisoprenaline (Powell & Slater, 1958), drugs have become available which specifically blocked β -adrenoceptors and methods for studying the properties of this group of drugs in man have been sought.

The assessment of β -adrenoceptor blockade

Resting heart rate

In normal subjects, heart rate is under the control of both the sympathetic and parasympathetic nervous systems (Robinson, Epstein, Beiser & Braunwald, 1966). Studies using either atropine or propranolol or combinations of both drugs have indicated that, at rest, the predominant influence on heart rate is parasympathetic activity. This influence may be attenuated in patients with cardiac disease (Eckberg, Drabinsky & Braunwald, 1971).

Thus, although most β -adrenoceptor blocking drugs slow resting heart rate, they provide no quantitative measure of receptor antagonism. This is due not only to the small amount of sympathetic activity normally present under resting conditions but also to the partial agonist activity which only some of these drugs possess.

Two examples may be given of the inaccurate results obtained by the measurement of β -adrenoceptor antagonism by effect on resting heart rate. Jackson, Atkinson & Oram (1975) described twenty-one patients with angina pectoris treated with propranolol or tolamolol who were classified as treatment failures because of a reduction in resting heart rate to 55–60 beats/min without symptomatic improvement. These subjects were re-evaluated using peak exercise heart rate as an index of β -adrenoceptor antagonism and resting heart rate was ignored. The dose of β -adrenoceptor blocking drugs was increased to produce exercise heart rates of 100 beats/min or less or a consistent rate of 100–125 beats/min despite

Table 1 Distribution of adrenoceptors

Organ	Receptor	Effect of stimulation
Heart	β_1	Increase in heart rate
	β_1	Increase in cardiac contractility
	β_1	Accelerate A-V conduction
Bronchi	β_2	Dilatation
Blood vessels	β_2	Dilatation
	α	Constriction
Eye	α	Dilatation of pupil
Gastrointestinal tract	α and β	Reduction in motility

progressive dose increments. With this new technique, fifteen of the twenty-one patients were subsequently considered to have responded satisfactorily to medical treatment. Secondly, for some years β -adrenoceptor blocking drugs were assessed in hyperthyroid patients by their effect on resting tachycardia. On this basis, it was found that a significant relationship between dose and effect could be obtained by drugs without partial agonist activity but not by those with this property (Turner, 1974) and the latter drugs were thought to be unlikely to be effective in this disease situation. However, Carruthers, Ghosal, McDevitt, Nelson & Shanks (1974) showed that, with a modified exercise test, a significant dose-response relationship could be obtained in hyperthyroid patients with practolol (a drug with partial agonist activity) and subsequently practolol was shown to be almost as effective as propranolol in the clinical management of this type of patient (Nelson & McDevitt, 1975).

Since β -adrenoceptor blocking drugs are competitive inhibitors, it is desirable to investigate their effects in the presence of increased adrenergic activity. Various methods of accomplishing this have now been evolved (Table 2). These procedures result in several responses, but as changes in heart rate are simplest to measure these have formed the basis of most tests of β -adrenoceptor function. The principle

Table 2 Methods of assessing β -adrenoceptor blockade in man

Isoprenaline tests
(a) Standardized bolus injections
(b) Continuous infusion
Exercise test
Glyceryl trinitrate test
Tilt test
Valsalva's manoeuvre
Standing upright

exception is in tests for cardioselectivity which will be discussed separately.

Isoprenaline test

Isoprenaline is the most potent activator of β -adrenoceptors known and also has little effect on α -receptors and so it was used for testing β -adrenoceptor blocking drugs in animals from the outset (Shanks, 1966). Isoprenaline was given intravenously and the response measured prior to and after administration of the antagonists under study.

This technique was subsequently transferred to the investigation of these drugs in man. Whether it was understandable caution at applying new methods to man involving drugs that were not without potential hazard or an improper appreciation of the underlying pharmacological principles, isoprenaline or other catecholamines were initially given as fixed doses, employing only one or at most two concentrations of the agonist. However, in any individual the response to a fixed dose of agonist varies in a normally distributed fashion and the accuracy of a single measurement is comparatively low (George, 1975). In addition the response to a small fixed dose of isoprenaline may be abolished for several hours after an oral dose of propranolol (Patterson, Conolly, Dollery, Hayes & Cooper, 1970). Using this type of methodology, claims were made that β -adrenoceptors were abnormally sensitive in patients with 'hyperkinetic hearts' (Frolich, Tarazi & Dustan, 1969) and were hyporesponsive in patients with asthma (Cookson & Reed, 1963). This resulted in criticism of tests of isoprenaline sensitivity on the grounds of use of fixed doses, failure to standardize doses of isoprenaline or to distinguish reflex changes in heart rate due to the fall in blood pressure from those due to direct β -adrenoceptor stimulation (Bourne, Thomson & Melmon, 1970).

Standardized bolus injections

Subsequently and almost simultaneously, two groups of investigators developed standardized isoprenaline sensitivity tests based on the measurement of log dose-response curves to rapid intravenous injections of isoprenaline (Cleaveland, Rangno & Shand, 1972; George, Conolly, Fenyyesi, Briant & Dollery, 1972). The techniques reported were almost identical.

The basis of the test is the rapid injection of increasing doses of isoprenaline into a freely running intravenous saline or glucose infusion and measurement of the maximum effects that these have on resting heart rate. The subjects lie supine in a quiet room, having been previously informed of the likely subjective effects, in particular the intense desire to inhale that occurs after isoprenaline administration,

and instructed to try and breathe as normally as possible. The starting dose of isoprenaline is 0.1 to 0.25 μg and subsequently successive doses are doubled until an increase of 30 to 35 beats/min results. Heart rate is allowed to return to normal between doses. The number of doses required to construct the dose-response curve varies from three to more than five but the duration of the test is usually not more than 40 minutes. The test may be repeated after a β -adrenoceptor blocking drug has been given, resulting in a parallel shift to the right of the isoprenaline dose-response curve (indicating competitive antagonism) and the effect of the antagonist quantified as the dose ratio (i.e. the dose required to increase the heart rate by 25 beats (CD_{25}) after propranolol divided by the CD_{25} before propranolol).

Several general points may be made. The presence of sinus arrhythmia may make the measurement of maximum heart rate response more difficult because of variations in resting heart rate, and Cleaveland *et al.* (1972) have shown that the dose-response curves and the CD_{25} calculated from mean resting rate, peak resting rate or following atropinization may differ. However, the effects are minimized by calculating from peak resting heart rate and by using an increase response of 25 beats/min rather than a smaller level (e.g. CD_{15}). It appears that the technique is highly reproducible and consistent within each subject when repeated several times within hours and even over a period of 6 months. Cleaveland and his colleagues (1972) found that the coefficient of variation in five subjects averaged 19.8%. However, there was considerable between subject variation, which existed even when correction was made for weight differences (George *et al.*, 1972). It did not appear that the CD_{25} was influenced by sex, weight, surface area or resting or post-atropine heart rates but it was significantly related to age, between 21 and 35 years (Cleaveland *et al.*, 1972). In a later study in normal subjects and hypertensive subjects, it has been suggested that the CD_{25} also correlates with basal diastolic arterial pressure (London, Safar, Weiss & Milliez, 1976).

Overall then a standardized isoprenaline test of this type has allowed accurate assessment of β -adrenoceptor blockade to be made in man provided attention is given to experimental detail. It has the advantage of being suitable for use in patients unable to undertake severe exercise. It is both qualitative and quantitative in that a parallel shift to the right of the dose/response curve in the presence of a β -adrenoceptor blocking drug suggests competitive antagonism. Calculation of the dose ratio allows the effects of varying doses of β -adrenoceptor antagonists to be measured accurately, and this estimate of pharmacological effect has been found to correlate with plasma drug levels (Coltart & Shand, 1970).

Against that, intravenous isoprenaline dose-response curves are non-physiological and hence provide no information concerning the effects of β -

adrenoceptor blocking drugs on endogenous sympathetic activity. In addition, patients with ischaemic heart disease may be put to unnecessary risk by the administration of isoprenaline intravenously. Cleaveland *et al.* (1972) found ventricular ectopic beats following the largest doses of isoprenaline in two of the fifty-eight tests they performed in normal subjects. These were associated with heart rate increases of 40 to 42 beats/min and for this reason they recommended that increases of no greater than 30–35 beats/min and absolute heart rates of about 145 beats/min should be aimed at. Lastly measurement of the effects of isoprenaline boluses on blood pressure cannot easily be made unless an arterial catheter is introduced and routine invasion of this type would defeat the simplicity and the relative lack of inconvenience to the patient.

Continuous isoprenaline infusion

Another method by which log dose-response curves have been produced is by the continuous intravenous infusion of isoprenaline with incremental increases in the infusion concentration, once steady state levels have been achieved. This certainly offers the advantage of allowing more accurate measurements of parameters other than heart rate—for example, blood pressure by sphygmomanometry and respiratory function by peak flow rate, spirometry or body plethysmography. The disadvantages include more prolonged exposure of subjects to the unpleasant side-effects of isoprenaline and a likelihood of relatively greater increases in heart rate as the experimental design is linked to dose rather than to effect. In addition, Briant, Dollery, Fenyvesi & George (1973) have suggested that, although the tachycardia produced by rapid injections is mainly the result of β -adrenoceptor stimulation (Cleaveland *et al.*, 1972), infusions of 3 min or more may result in a substantial reduction in vagal tone. There has also been some suggestion that prolonged infusion of isoprenaline may produce tolerance (Conolly, Davies, Dollery & George, 1971) but this is not universally accepted.

Generally then, log dose-response curves derived from bolus injections of isoprenaline are preferable to incremental infusions but both have their limitations in relation to the subjects in which they may be performed and the interpretation of results compared with effects on the endogenous sympathetic nervous system.

There is another problem that occurs with isoprenaline, related to observed differences between some of the β -adrenoceptor antagonists. It can be shown that different β -adrenoceptor blocking drugs in doses which produce approximately equal effects on exercise tachycardia in man may not be equipotent when tested by isoprenaline stimulation. This is believed to be due to the fact that the increase in heart

rate in response to isoprenaline results from two mechanisms—a direct effect of the drug on the sino-atrial node and a reflex reduction in vagal inhibition in response to the fall in arterial pressure produced by isoprenaline (Dunlop & Shanks, 1968). Thus drugs which block β_1 - and β_2 -adrenoceptors, like propranolol, abolish both components and may completely prevent the increase in heart rate. On the other hand, cardioselective drugs like practolol block the effect on the sino-atrial node but do not effect the reflex reduction in vagal inhibition. Partial confirmation for this comes from one of the first studies on practolol in man. It was shown that the effectiveness of practolol in reducing an isoprenaline tachycardia was improved by the prior administration of atropine (Brick, Hutchinson, McDevitt, Roddie & Shanks, 1968). Unfortunately single doses only of isoprenaline were used. However, it would appear that direct comparisons of the efficacy of β -adrenoceptor antagonists, where one or more of the drugs may be cardioselective, cannot be made by isoprenaline stimulation unless prior atropinization is undertaken. It has been suggested that this problem applies only to isoprenaline infusions and not to the tachycardia produced by rapid injections (Briant *et al.*, 1973) with which these authors reported parallel shifts to the right of dose-response curves with practolol. However, they did not examine the effects of prior atropinization on the dose-response curves with a cardioselective drug and final proof for this claim must, therefore, be regarded as lacking.

Exercise testing

As previously described, resting heart rate is predominantly influenced by the parasympathetic nervous system. The tachycardia of exercise is produced by a combination of sympathetic stimulation and parasympathetic withdrawal (Robinson *et al.*, 1966). Robinson, Percy, Brueckman, Nicholas & Miller (1953) have shown that exercise heart rates of up to 200 beats/min are necessary before vagal influence is completely withdrawn. Such levels of exercise are difficult to attain repeatedly in normal subjects and certainly impossible in patients with ischaemic heart disease or hypertension. However a substantial sympathetic component can be expected with exercise heart rates of lesser degree. George (1975) recommends exercise to produce heart rates of at least 130 beats/min and, in normal subjects, levels of 160 beats/min or greater are desirable. In patients with severe ischaemic heart disease, exercise tachycardias of 140 beats/min or greater have been described in the assessment of β -blockade (Jackson *et al.*, 1975).

Various methods of standardized exercise have been employed to produce these levels of heart rate in the clinic and laboratory situation. Those most

commonly employed are step tests, bicycle ergometry and treadmill exercise. Difficulty may arise where these tests involve sustained hand grip, particularly likely with erect bicycle ergometry, as it has been shown that this produces a rise in heart rate (Lind, Taylor, Humphreys, Kennelly & Donald, 1964) and that this may increase after propranolol is injected, possibly due to relative vagal inhibition (Macdonald, Sapru, Taylor & Donald, 1966). Such an independent variable may confuse a standardized test.

Consideration must be given to the problems of repeated testing to compare the effects of different doses or drugs or for studies on the duration of action. This has proved possible in normal subjects with a step test which takes 3 min only to complete. However, in comparative studies involving several doses of more than one drug the duration of the experiment may stretch into weeks or even months. Therefore, the dose-response curves with sotalol and a step test were studied using five subjects who were each given five doses of the drug and thirty subjects who each received a single dose at six different dose levels. The dose response curves obtained were very similar (Brown, Carruthers, Kelly, McDevitt & Shanks, 1976). It would appear then that provided the exercise test is standardized and exercise effects are presented in a manner suitable for intersubject comparison, general comparisons between drugs may be valid without the ultimate stringency of within subject testing.

There has been a lack of agreement about how the effects of a drug on heart rate should be presented. Thus measurements of β -adrenoceptor blockade include comparison of the absolute heart rate before and after drug administration (Brick *et al.*, 1968), the percentage inhibition of exercise tachycardia (Fitzgerald & Scales, 1968), the percentage blockade of sympathetic activity (Coltart & Shand, 1970), the reduction of control exercise heart rate (Bodem & Chidsey, 1973) and the percentage reduction of exercise heart rate (Carruthers, Kelly, McDevitt & Shanks, 1974). Confusion has resulted from this lack of standardization and made results from different studies difficult to compare.

An attempt has recently been made to evaluate these methods of expressing the effects of β -adrenoceptor antagonism on exercise heart rate (Carruthers, Shanks & McDevitt, 1976). This necessitated investigation of the 'intrinsic heart rate on exercise' so that true sympathetic activity could be calculated. The effects of drugs could then be expressed as percentage blockade of sympathetic activity and this figure compared with the other methods of presentation outlined above.

Chamberlain, Turner & Sneddon (1967) have shown that maximum inhibition of the vagal control of the sinoatrial node in normal subjects can be achieved both at rest and during exercise by the administration of atropine 0.04 mg/kg intravenously. Using this dose

of atropine combined with propranolol 0.2 mg/kg intravenously, Jose (1966) demonstrated that in man the chronotropic response of the heart was totally blocked to vagal or sympathetic stimuli. The resultant measurement he called 'the intrinsic heart rate', indicating freedom from the influence of the autonomic nervous system. However, his subjects were challenged by isoprenaline but not by severe exercise.

With standardized exercise, Carruthers *et al.* (1976) have shown that the vagal component appears reproducible in the same individual. Once this vagal component has been quantitated, calculations of 'intrinsic heart rate on exercise' or sympathetic activity can be made without prior atropinization. It was also concluded that changes in exercise heart rate after β -adrenoceptor blockade can be attributed to alteration in sympathetic activity alone rather than possible combined alterations in both the sympathetic and parasympathetic nervous systems.

'Intrinsic heart rate on exercise' differed from 'intrinsic heart rate at rest', requiring propranolol 0.2 to 0.6 mg/kg intravenously or greater, in addition to atropine 0.04 mg/kg to achieve near complete autonomic blockade. In addition 'the intrinsic rate on exercise' was always higher in any individual than that recorded at rest and the differences between the two were significant. Why the heart rate increases with exercise after autonomic blockade is not clear but has been seen in greyhounds after cardiac transplantation and bilateral adrenalectomy (Donald, 1974) and appears to be independent of catecholamines.

The sympathetic component of exercise in these six subjects varied from 39–75 beats/min and the effects of practolol from a previous study with the same subjects (Carruthers *et al.*, 1974) could then be expressed as percentage blockade of total sympathetic activity and compared with other conventional methods of assessing β -adrenoceptor blockade. This showed that absolute reduction ($r=0.78$) and percentage reduction ($r=0.81$) of exercise heart rate demonstrated the best relationships with the specific measurement of sympathetic blockade. Absolute heart rate ($r=0.48$) and percentage inhibition of tachycardia ($r=0.62$) also correlated significantly, but less well. Calculation of absolute reduction and percentage reduction of exercise heart rate depend only on knowledge of the control exercise heart rate and the exercise heart rate after the administration of β -adrenoceptor antagonists, without knowledge of resting heart rates or total sympathetic activity. These simple methods are clearly the most suitable at this level of exertion.

Exercise testing as a means of assessing β -adrenoceptor antagonism is, therefore, extremely useful, can be done simply and quickly with a minimum of equipment, and has the additional advantage that it measures the effects of an endogenous physiological stimulus.

Glyceryl trinitrate test

The tachycardia which follows the sublingual administration of glyceryl trinitrate has been attributed to a reflex response to the fall in arterial blood pressure, and Wiener, Dwyer & Cox (1969) reported that they had blocked a trinitrin tachycardia in ten supine patients with angina by the administration of propranolol (10 mg intravenously). Fitzgerald (1970) suggested, therefore, that the positive chronotropic effects of trinitrin were due solely to a sympathetically mediated reflex and that this reflex could be used to assess β -adrenoceptor blockade.

The principle of the test was to administer glyceryl trinitrate 0.5 mg sublingually to a subject in the upright position, to follow the heart rate for 6 min after drug was given by means of a cardi tachometer or electrocardiograph and to take the highest heart rate obtained in any one minute as the maximum response. Fitzgerald (1970) showed that the tachycardia obtained was reproducible within the same individual, was unaltered by the administration of atropine (2.5 mg intravenously) and was reduced by giving both propranolol and practolol. Significantly greater tachycardia occurs in the erect than in the supine position (Kofi Ekue, Shanks & Walsh, 1974).

There is doubt as to whether the reflex is mediated entirely by the sympathetic nervous system. Fitzgerald (1970) found that the trinitrin tachycardia was not abolished either by propranolol (640 mg) or by practolol (1200 mg), but felt that this was due to incomplete blockade. However in two subsequent series (Kofi Ekue *et al.*, 1974; Zweifler & Hansson, 1976) the original trinitrin tachycardia was not completely obliterated by the exhibition of β -adrenoceptor antagonists in increasing doses, and it was felt that more than sympathetic stimulation was involved. The lack of effect of atropine does not completely exclude the possibility that parasympathetic influences may operate after the administration of β -adrenoceptor blocking drugs. Combined parasympathetic and sympathetic blockade would be required to elucidate this point but has so far not been attempted.

However, although the increase in rate probably does not result entirely from increased sympathetic activity, the glyceryl trinitrate test is of some value in the assessment of β -adrenoceptor blockade, both in normal subjects and in patients with ischaemic heart disease (Kofi Ekue *et al.*, 1974) and hypertension (Zweifler & Hansson, 1976). In one series it correlated well with responsiveness to isoprenaline (Zweifler & Hansson, 1976) in another standing tachycardia (Kofi Ekue *et al.*, 1974). Occasional subjects feel light-headed or faint after taking GTN in the erect position. It is a test of whether some degree of β -adrenoceptor blockade exists but as dose-response curves cannot be constructed and as some patients object to the side-effects it has never achieved wide usage. In the patient

study by Kofi Ekue *et al.* (1974), standing gave similar results and was considered preferable.

Other methods

Various other methods have been described by which β -adrenoceptor blockade can be assessed. These include tilting, Valsalva's manoeuvre or standing. The tachycardia produced by upright tilting has been shown to be reduced by β -adrenoceptor antagonism and abolished by combined blockade with propranolol and atropine (Robinson *et al.*, 1966). However, not only does tilting appear to produce a comparatively weak stimulus, the sympathetic component of which may be abolished by even modest doses of propranolol (George, Fenyvesi & Dollery, 1973) but, in addition, the response seems to vary diurnally and in relation to the degree of hydration (Abelmann & Fareeduddin, 1969). Predictability and repeatability are, therefore, uncertain, dose-response curves are difficult to obtain and special apparatus is required. For these reasons the test has been little used. Similarly Valsalva's manoeuvre and standing upright from a supine position have not been widely employed. However, in several clinical studies standing has been shown to produce assessments of β -adrenoceptor blockade not dissimilar from those obtained by more elaborate methods—severe exercise or glyceryl trinitrate tachycardia (Carruthers *et al.*, 1974; Kofi Ekue *et al.*, 1974). Such a simple method may be valuable in the outpatient clinic to assess whether sympathetic blockade exists, but quantitatively the information may be unreliable.

In conclusion, of the various techniques available for the assessment of β -adrenoceptor blockade in man, all are subject to limitations of applicability, information obtainable and interpretation. Isoprenaline induced tachycardia and exercise testing have been most widely employed and the latter certainly has the advantage of being more physiological, and using endogenous sympathetic stimulation. The level of

exercise required to make the test meaningful may, however, preclude its use in patients with certain types of diseases. Simpler tests like moderate exercise or standing upright may yield sufficient information if quantitative results are not required.

In addition to their ability to block β -adrenoceptors, these drugs have been shown in animals to have additional properties—membrane stabilizing activity, cardioselectivity and partial agonist activity. The distribution of these properties in relation to the commonly available β -adrenoceptor blocking drugs is shown in Table 3.

Membrane stabilizing activity

Morales-Aguilera & Vaughan Williams (1965) demonstrated, in animals, that pronethalol had a quinidine-like effect on the heart and that it was a potent local anaesthetic. Both these properties are included in the term 'membrane stabilizing activity', and there has been a great deal of controversy about its contribution to the effects of β -adrenoceptor blocking drugs in angina pectoris and in cardiac arrhythmias in man. However, the membrane stabilizing effect of propranolol has been demonstrated *in vitro* with human muscle only at a minimum concentration of 10 mg/l (Coltart & Meldrum, 1970) which is about 100 times greater than that required for inhibition of exercise tachycardia or suppression of ectopic beats (Coltart & Shand, 1970; Coltart, Gibson & Shand, 1971). This is, therefore, not thought to be an important property of β -adrenoceptor blocking drugs in the doses used in clinical practice and there are no *in vivo* methods for demonstrating it in man.

Cardioselectivity

The subdivision of β -adrenoceptors into β_1 and β_2 was supported by the development of practolol which, in animals, inhibited the effect of isoprenaline on the

Table 3 Properties of β -adrenoceptor blocking drugs

Drug	Proprietary name	β -Adrenoceptor blockade	Membrane stabilizing activity	Cardioselectivity	Partial agonist activity
Acebutolol	Sectral	+	+	—	—
Alprenolol	Aptin	+	+	—	+
Atenolol	Tenormin	+	—	+	—
Metoprolol	Lopressor	+	+	+	—
Oxprenolol	Trasicor	+	+	—	+
Pindolol	Visken	+	+	—	+
Practolol	Eraldin	+	—	+	+
Propranolol	Inderal	+	+	—	—
Sotalol	Beta-cardone	+	—	—	—
	Sotacor	+	—	—	—
Timolol	Blocadren	+	+	—	—

heart but had little effect on vasodilatation and bronchodilatation (Dunlop & Shanks, 1968). Practolol as a β -adrenoceptor antagonist was, therefore, said to be cardioselective and similar properties have subsequently been claimed for other drugs, including acebutolol, atenolol, and metoprolol, on the basis of animal studies.

Assessment of cardioselectivity in man is not easy and has been misunderstood. Ideally, such a claim must be based on demonstrating that the drug blocks the effect of an agonist e.g. isoprenaline, on the heart while having minimal effects on the bronchodilator and vasodilator responses. Measurement of the effects of β -adrenoceptor antagonists on resting respiratory function either in normal or asthmatic subjects does not fulfill these criteria and cannot be accepted as evidence of cardioselectivity important though it may be in the clinical context. It is akin to measuring the cardiovascular effects of these drugs by changes in resting heart rate, the fallacious results of which have already been discussed. Thus methods of stimulating both types of β -adrenoceptors are required where simultaneous measurement of the effects of antagonists can also be made.

The first attempt to demonstrate cardioselective properties in man was made by Brick *et al.* (1968) when studying practolol. They studied the effects of a single dose of isoprenaline by continuous infusion on heart rate, forearm blood flow and arterial blood pressure (measured via a brachial artery catheter) both before and after the intravenous administration of propranolol and practolol in normal subjects. Propranolol prevented the increase in heart rate and forearm blood flow and the decrease in diastolic blood pressure which isoprenaline caused. Practolol had little effect on the isoprenaline induced changes in diastolic blood pressure and forearm blood flow, but although it reduced the isoprenaline tachycardia, the change was not significant unless atropine was given first. Reference has previously been made to the possible role of reflex vagal tachycardia after vasodilatation and to the likely different effects, therefore, of selective and nonselective β -adrenoceptor antagonists. Thus this technique is only valid if subjects are pre-treated with atropine and cannot be regularly repeated. Its other disadvantage was that no information about the respiratory receptors was provided. Nevertheless, despite these objections, and the use of only a single concentrations of isoprenaline, this original study did suggest that practolol might be cardioselective. A modification of these techniques was later employed by Briant *et al.* (1973). Measuring similar parameters, they used a combination of continuous intra-arterial infusions and intravenous injections of isoprenaline and were able to produce limited dose-response curves with roughly parallel shifts to the right compared to the more shallow slopes of post-practolol dose-response curves reported after isoprenaline infusions. Some of the objections outlined

above may, thus, be overcome by these modifications.

A further method for investigating cardioselectivity in patients with obstructive airways disease was suggested by Powles, Shinebourne & Hamer (1969). They measured resting heart rate and forced expiratory volume in one second (FEV₁) before and after four full inspirations of a 4% isoprenaline aerosol. These measurements were then repeated after the intravenous administration of practolol. It was found that the practolol blocked the isoprenaline-induced tachycardia seen in the control situation whilst the bronchodilator response to isoprenaline was identical before and after the β -adrenoceptor antagonist—that is, whilst the cardiac β -adrenoceptors were blocked, the respiratory receptors were not, a clear demonstration of cardioselectivity. Despite the simplicity and efficacy of this technique, it seems to have been utilized infrequently.

Recently Kumana, Marlin, Kaye & Smith (1974) have shown that peak flow rate (PFR) increases with severe exercise in normal subjects. This has been used as a basis for assessing the cardioselectivity of β -adrenoceptor blocking drugs. Subjects undertake severe exercise under placebo control and after the administration of the drug or drugs to be tested and several measurements of exercise heart rate and of exercise peak flow rate can be made during the period after the drug has been given. The simultaneous effects of the drug on cardiovascular and respiratory function can then be compared with the control results and observations on cardioselectivity made. Initially Kumana *et al.* (1974) compared propranolol, practolol and a placebo using this method, following the effects for 24 hours. They were able to show that both active drugs had a significantly greater effect on exercise heart rate than did placebo. Also, although propranolol significantly reduced PFR compared to the placebo, practolol did not. This appeared to be a satisfactory response, but unfortunately comparison of the effects of propranolol and practolol on exercise PFR showed a significant difference only at 6 hours. This appeared rather equivocal and a subsequent study comparing propranolol, practolol and acebutolol also gave results which were difficult to interpret in terms of cardioselectivity (Kumana, Kaye, Leighton, Turner & Hamer, 1975). Thus, although theoretically this technique has much to commend it, in practice the results have been disappointing and it has the added disadvantage that it is not suitable for patients with obstructive airways disease.

Variations or combinations of these methods have also been described. Vilsvik & Schaanning (1976) have compared the effects of practolol and atenolol, both reputedly cardioselective, in patients with asthma by means of exercise tachycardia up to a mean level of 130 beats/min followed 45 min later by inhalation of isoprenaline sulphate 1500 μ g. They measured heart rate, blood pressure, FEV₁ and PFR and showed that practolol and atenolol performed comparably,

indicating that atenolol was at least as cardioselective as practolol. Ideally, however, one would want to show that a drug which was not cardioselective e.g. propranolol, performed differently within the system, even though this would necessitate some minor inconvenience to patients with obstructive airways disease. In addition, the authors intended that the exercise test should investigate cardiac blockade and the isoprenaline test effect on bronchial receptors. As the tests are separated by 45 min and the elimination rate of the drugs may be different, simultaneous equality of cardiac response compared to inequality of respiratory response must be inferred.

Isoprenaline testing has recently been extended to include measurement of respiratory function for the assessment of cardioselectivity. Graded doses of isoprenaline by continuous intravenous infusion have been employed in asthmatic patients in whom FEV₁, heart rate and blood pressure by sphygmomanometry have been recorded (Johnsson, Svedmyr & Thiringer, 1975; Thiringer & Svedmyr, 1976). In both studies metoprolol and propranolol were compared; in one of them, practolol was also included. The difficulties associated with measuring isoprenaline-induced tachycardia after continuous infusion were countered by using doses of the drugs previously shown to be equipotent by exercise tachycardia and by comparing metoprolol (the drug whose properties were principally under investigation) with practolol. It was shown that propranolol almost completely blocked the effects of all doses of isoprenaline on heart rate and FEV₁, and, to a lesser extent, any changes in systolic and diastolic pressure. Metoprolol and practolol did not inhibit the effect of isoprenaline on FEV₁, whereas their effect on the isoprenaline-induced increase in heart rate indicated a selectivity for β_1 -receptors. Use of this technique enables comprehensive and comparative information to be provided between several β -adrenoceptor antagonists. Infusion of isoprenaline allows accurate recording of respiratory function and blood pressure without complicated or invasive techniques and distortion of heart rate effects can be compensated for as outlined above. It has the additional advantage of use in patients who are likely to require selective drugs in clinical practice.

It should be emphasized that cardioselectivity is a relative rather than a specific property. Not only is the response of patients variable—that is, some patients with airways obstruction may not be made clinically worse by the administration of non-selective drugs, whereas others will wheeze with cardioselective agents—but animal studies have always suggested that selectivity may be a dose-dependent phenomenon. Recently it has been demonstrated that, in normal subjects, practolol at a dose of 1.5 mg/kg orally significantly reduced exercise tachycardia but did not attenuate the forearm vasodilator responses to isoprenaline. When the dose of practolol was increased to 12 mg/kg, both the exercise tachycardia

and the forearm vasodilator response to isoprenaline were significantly reduced (Lertora, Mark, Johannsen, Wilson & Abboud, 1975). Cardioselectivity with practolol would, therefore, appear to have been lost between approximate doses of 100 mg and 800 mg. Considering the large doses of β -adrenoceptor antagonists sometimes employed in clinical practice, this is a possible consequence worth remembering.

Lastly, it must be stressed again that whilst effects of β -adrenoceptor antagonists on resting respiratory function may be clinically important, claims for cardioselectivity of drugs should not be based on them. None of the methods outlined is without its problems and difficulties but, despite this, reasonably reliable information about the cardioselectivity of β -adrenoceptor blocking drugs has now become available. Whilst uncertainty about methodology remains it is important that definitive studies of new drugs should include evidence of the effect of known agents, both selective and non-selective, as a safeguard to the veracity of any conclusions. Secondly, comparison between drugs as to their relative cardioselectivity must be done with doses and at times when equal effect on cardiac receptors can be demonstrated.

Partial agonist activity

β -Adrenoceptor antagonists may have sympathomimetic effects in doses which produce β -blockade. The intrinsic activity of the drug receptor complex is still sufficient to cause agonist activity even though the responses to other agonists are blocked.

This property has been called partial agonist activity and can be demonstrated both in isolated atrial preparations (Blinks, 1967) and in intact animals depleted of their noradrenaline stores by pretreatment with reserpine or syrosingipine (Dunlop & Shanks, 1968). In cats pretreated with syrosingipine, dichloroisoprenaline and pronethalol increased heart rate by 97 and 24 beats/min respectively, whereas propranolol had no effect. The increases were attributed to partial agonist activity.

It can be seen from Table 3 that some β -adrenoceptor blocking drugs have partial agonist activity and others do not. This refers to the demonstration of this effect in animals. Its demonstration in man has proved more difficult, nor is it clear whether this property has any clinical significance.

Two possible indicators of partial agonist activity in man have been described. Firstly, as already indicated, β -adrenoceptor blocking drugs with this property may reduce resting heart rate less than those without it. Secondly, flattening of the dose-response curve with exercise at higher doses has been reported with drugs with partial agonist activity (Carruthers *et al.*, 1974; Harry, Knapp, Linden, Newcombe & Stoker, 1975).

Recently in comparing the dose-response curves obtained with five different β -adrenoceptor antagonists with an exercise test, it has been pointed

out that two distinct types of response appear to be seen (McDevitt, Brown, Carruthers & Shanks, 1977). In the first, increasing doses of the β -adrenoceptor antagonist continue to produce increasing effect, certainly to the limit of the dose levels examined. Drugs producing this type of response are sotalol and propranolol, and the resultant dose-response curves appear to be parallel. Secondly, with some drugs, increasing the dose initially results in substantial increase in effect but subsequently larger doses produce almost no further reduction in heart rate. Oxprenolol and practolol are drugs in this category and these dose-response curves also appear to be similar to one another. The common additional property which these two drugs have which is not shared by propranolol and sotalol is partial agonist activity and it seemed possible that this was influencing the curves. However, practolol is also cardioselective and the atenolol dose-response curve appeared flat in the middle dose range so this property may have contributed.

Administration of combinations of β -adrenoceptor blocking drugs has been used to try and clarify the situation (McDevitt *et al.*, 1977). Sotalol was chosen because it has no additional properties, oxprenolol because it has partial agonist activity, atenolol because it is cardioselective and practolol because it has both partial agonist activity and cardioselectivity. Groups of four subjects were given combinations of sotalol and one of the other drugs, one at time zero and the 2 h later. Exercise tests were performed at +2 h, before the second drug was given, and at +4 h, 2 h after administration of the second drug. A Latin Square design was used so that each subject had each of the four possible combinations of the two drugs. Approximately equipotent doses of each drug were given.

The results showed that sotalol given as the second drug after sotalol, atenolol, oxprenolol or practolol produced a further significant increase in the percentage reduction of exercise heart rate. Similarly atenolol given after either atenolol or sotalol also resulted in further significant effect. However, the administration of oxprenolol after either sotalol or oxprenolol, or practolol after either sotalol or practolol did not have any significant effect. Partial agonist activity, then, rather than cardioselectivity appeared to be the significant factor.

It would appear from these studies that flattening of the dose-response curves with an exercise test does occur with some drugs, and, that the diminished responses obtained at higher doses with drugs such as oxprenolol and practolol can be improved by giving an additional drug like sotalol but not by increasing the original compound. The similarity in performance between oxprenolol and practolol on the one hand and sotalol and atenolol on the other would suggest that the presence or absence of partial agonist activity is the important difference between these two groups of drugs, and that this additional property is

demonstrated by flattening of the dose-response curve on exercise, where, at higher doses the partial agonist activity modifies the capacity to competitively block β -adrenoceptors. It would appear also, on the basis of these results, that the very large doses of β -adrenoceptor antagonists sometimes employed are unlikely to be more effective than much smaller doses in terms of β -adrenoceptor blockade when the drug has the additional property of partial agonist activity.

Pharmacological effects of β -adrenoceptor blocking drugs in relation to plasma levels

Generally, good correlation can be shown between the effects of β -adrenoceptor antagonists and the logarithms of blood or plasma concentrations (Coltart & Shand, 1970; Ablad, Ervik, Hallgren, Johnsson & Solvell, 1972; Carruthers *et al.*, 1974; Cuthbert & Collins, 1975; Brown *et al.*, 1975; Brown, Carruthers, Johnston, Kelly, McAinsh, McDevitt & Shanks, 1976). Nevertheless, several peculiarities require further explanation.

It has been shown that, although there was a straight line relationship between the block of exercise tachycardia and the logarithm of plasma propranolol concentration, the propranolol level associated with a given effect 2 h after a single oral dose was one half that required after intravenous administration (Coltart & Shand, 1970)—that is, oral propranolol appeared to be twice as effective as intravenous. An explanation for this apparent discrepancy is provided by the work of Paterson *et al.* (1970). They showed that an active metabolite of propranolol, 4-hydroxy propranolol, can be detected in the plasma only after oral administration. This metabolite is not measured by the standard propranolol assay, but since it is equipotent to propranolol (Fitzgerald & O'Donnell, 1971) and achieves approximately the same circulating concentrations as the parent drug shortly after an oral dose (Paterson *et al.*, 1970), the effects of the metabolite will add to those of propranolol. The presence of this metabolite after oral but not intravenous propranolol would appear to account for the twofold difference in the effective propranolol concentration by the two routes of administration. However, 4-hydroxypropranolol has a shorter half-life than propranolol (Paterson *et al.*, 1970) so that even 6 h after a single oral dose, all of the effects can be accounted for by the parent compound (Cleaveland & Shand, 1972). This is also true on chronic oral administration, even 2 h after an individual dose, due to accumulation of propranolol.

While there is excellent correlation between plasma propranolol concentration and effect in any given individual, there is considerable variation among subjects in the effectiveness of the total plasma concentration. For example, George *et al.* (1973) found as much as four-fold difference in normals, and Zacest & Koch-Weser (1972) have described two populations of hypertensive patients, one of which

required 2.5 times the propranolol concentration to achieve comparable isoprenaline antagonism during chronic oral administration. Possible explanations for these differences include variation in the amount of 4-hydroxypropranolol production, individual differences in receptor sensitivity or the effect of known difference in plasma drug binding of propranolol.

The relationship of free and total propranolol concentrations to its effectiveness in antagonizing isoprenaline-induced tachycardia in both normal and hypertensive subjects has been recently investigated (McDevitt, Frisk-Holmberg, Hollifield & Shand, 1976). This was done by performing isoprenaline sensitivity tests both before and after steady-state concentrations of propranolol had been produced by continuous intravenous infusion. The effectiveness of propranolol was estimated as the dose ratio (DR) to isoprenaline: plasma drug binding was estimated by equilibrium dialysis using radioactively labelled propranolol. Over the relatively narrow range of total plasma concentrations of 22.5 to 50 ng/ml achieved, there was no correlation between (DR-1) and total plasma concentration in normal subjects ($r=0.28$; $P>0.1$), a positive correlation for hypertensive patients ($r=0.74$; $P>0.05$) but no correlation for the pooled data ($r=0.42$; $P>0.1$). On the other hand, when (DR-1) was plotted against free drug concentration, there was an excellent positive correlation which extrapolated close to the origin in normals ($r=0.86$; $P<0.01$), hypertensives ($r=0.95$; $P<0.001$) and in the total population ($r=0.89$; $P<0.001$). Thus the effect of propranolol is a function of free drug concentration according to classical receptor theory. The true affinity of propranolol for its receptor calculated from free drug concentration varied only two-fold; the apparent affinity constant was twice as variable. It seems, therefore, for propranolol that the contribution of individual variation in receptor sensitivity to differences in oral dosage requirement is minor compared to that of variations in bio-availability.

With other β -adrenoceptor blocking drugs, plasma binding is either lower or has not been determined. Similar effects have not, therefore, been demonstrated.

Despite the relationships already shown between plasma concentrations of propranolol and its effects, there have been suggestions that the time-course of propranolol may not always reflect the changes in plasma concentration. On the one hand, the duration of action of β -adrenoceptor blocking drugs has been described as appearing to be longer than would have been appropriate to their plasma elimination half-lives (George, Fenyvesi, Conolly & Dollery, 1972; Viljoen, Estafanous & Kellner, 1972; Nigam & Malhotra, 1973); on the other Perrier & Gibaldi (1974) have suggested, on theoretical grounds, that the action of propranolol is related to its concentration in a tissue pool that equilibrates only slowly with plasma.

This latter claim has recently been investigated by

examining the effects of propranolol on an isoprenaline-induced tachycardia (McDevitt & Shand, 1975). In three normal subjects a constant infusion of isoprenaline was administered at a rate designed to increase resting heart rate by 20 to 25 beats/minute. When the tachycardia was stable, a small dose of propranolol (0.1 mg) was given by rapid intravenous infusion and the heart rate response followed until it returned to pre-injection levels. It was found that the propranolol-induced inhibition of the isoprenaline tachycardia was maximum within 2-4 min of the injection and declined rapidly thereafter, returning to baseline within 10 to 15 minutes. Plasma concentrations of propranolol could not be measured after such a small dose but changes in propranolol concentrations were simulated using a 2-compartment open model (Riegelman, Loo & Rowland, 1968) with parameters derived from the data of Evans, Nies & Shand (1973). This showed that the drug's effectiveness closely followed the time course of plasma levels, but bore no relationship to those in the deep tissue pool. In fact, the effects had dissipated before levels in these tissues had reached their maximum. This certainly applies to the property of β -adrenoceptor blockade but it is still possible that other effects of the drug could have a slower onset.

Assessment of the later effects of β -adrenoceptor blocking drugs must take careful account of the method being used. According to the classical receptor theory (Goldstein, Aronow & Kalman, 1974), the effects of a competitive antagonist should be described by the equation

$$(\text{DR}-1) = KC$$

in which DR is the dose ratio for the antagonist, K its association constant and C its concentration. Thus a plot of DR-1 against plasma concentration should yield a straight line with a slope K. Such a relationship can in fact be shown to exist in subjects in whom standardized isoprenaline tests were performed before and at 15 min, 3 and 6 h after the intravenous administration of propranolol 20 mg (McDevitt & Shand, 1975).

In contrast, the reduction in any given response by a competitive antagonist (i) is a hyperbolic function with respect to antagonist concentration (Goldstein *et al.*, 1974):

$$\frac{i}{1-i} = \frac{C}{K}$$

In this case a plot of i against the logarithm of C should yield a straight line over the range of 20–80% of i . Again data from Coltart & Shand (1970), in which reduction in heart rate due to strenuous exercise before, 15 min, 1 and 4 h after the administration of propranolol 0.3 mg/kg intravenously, show that percentage reduction in exercise tachycardia was quite well related to the logarithm of plasma concentration.

It is clear that when effectiveness is measured in

terms of the antagonism of isoprenaline as (dose ratio-1), then plasma concentrations and efficacy fall in parallel. In this case drug concentration half-life and half-life of effects may be properly related as both parameters decline exponentially after distribution has occurred. This is not true when percentage reduction in exercise-induced tachycardia is considered. Rather, this measure of efficacy is a function of log concentration, so that during the late phase from 1 h onward when plasma concentrations fall as a single exponential, there is a linear relationship between percentage reduction in rate and time i.e. effective-

ness follows a zero order decline and does not allow derivation of pharmacological half-life.

Thus, both the early and late effects of propranolol on heart rate, appear to be related to its plasma concentrations, but it is quite clear that care must be taken to tailor comments regarding the duration of action of β -adrenoceptor blocking drugs within the limitations of the methods used. Confusion has arisen from failure to do this.

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