Screening Methods in Pharmacology

Edited by

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Volume II

Screening Methods in Pharmacology

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Preface

The second volume of "Screening Methods in Pharmacology" has the same basic purpose as Volume I, namely, to present sufficient practical information about techniques so that it would be possible for the reader, even with little experience, to establish a screening program for a particular pharmacological activity. The contributors to this volume have presented typical results obtained for selected reference compounds, which are intended to show the responses with a known substance and to guide the reader during the initial use of a test method so that he may select suitable doses of the reference drugs and may know the intensity of the response expected for a certain dose level.

Because the progress in developing methods has been so rapid since the appearance of the previous volume, it became impossible for one person to review the pharmacological literature. Thus, unlike Volume I, Volume II is a multiauthored, coedited work.

ROBERT A. TURNER

PETER HEBBORN

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Introduction

Numerous methods often exist for screening a series of compounds for a given pharmacological activity. Many, but not all, available methods are described in this volume. They have been selected because they are the most reliable, the simplest, and, in the opinion of the respective authors, the preferred of the available methods. The sensitivity of the assay procedure and the possibility of ranking the compounds that have proved clinical effectiveness are important factors in the selection of a screening method.

Those who have been involved with screening drugs for pharmacological activity for even a short time have realized that only a few in a group of substances have activity. An alternative situation exists if one has a group of compounds, all of which have varying degrees of activity. In both cases, the screening process is an attempt to identify, by one or more tests, those few substances which are gems among a group of pebbles.

Generally it is better to use a screening method which may give a few false positives rather than one which will yield some false negatives. If a substance has no true activity and is shown by a test to have activity, a false positive results. Sooner or later, as testing with the substance is continued, its inactivity will be revealed. Some time may be wasted in studying the compound, but in the end the investigator is not misled. On the other hand, a false negative may result in the removal of a substance from further study, so that its activity will remain forever undetected.

The developer of a new drug is always seeking a relation between

chemical structure and biological activity, which, if found, is rare and retrospective, rather than deductive. Sometimes structural changes in a molecule that appear minor cause unpredictable and extensive changes in the pharmacological activity, including loss of all activity and introduction of new side effects. Often the first member of a homologous series of compounds is the most active pharmacologically. Because the biological consequences of small changes in chemical structure are not understood, the structural changes cannot be programmed logically. New drugs of a unique character will probably be derived in the future from novel structures rather than from modifications of old structures, study of enzyme systems involved in the disease state, unexpected clinical observations, and an understanding of the metabolism of known, active drugs.

Experience and scientific intuition play their important roles. Screening efficiently for certain pharmacological activities is necessary for progress. Since activity is unpredictable, the number of activities covered by the screening program should be considerable. If several tests have indicated that a compound has some activity, it is usually advantageous to study it further rather than to start with a new compound *ab initio*. Contemporary investigators of new drugs tend to screen with a broad program.

No procedure for screening can be perfect. Therefore, anyone performing screening in pharmacology should always be vigilant for borderline results and for results indicating an inactive substance when one strongly suspects that activity is present. If one has good theoretical grounds for anticipating activity of a substance, one should continue to study it, even if one screening procedure indicates that activity of a certain kind is absent. One should not rigidly accept the results of screening procedures, if, by doing so, one would relegate to the shelf a substance which might be valuable clinically.

It is possible for a drug to be metabolized or eliminated very rapidly by laboratory animals and yet to have a prolonged half-life in man. Phenylbutazone is an example of a drug having antirheumatic activity in man, but whose activity as an antiinflammatory agent in rodents is demonstrable only at doses approaching a lethal level. Moreover, in some disease states, available, clinically effective drugs are only palliative and not curative. It is reasonable to conclude that pharmacological screening tests in which such clinically active drugs have a positive effect can be used to select new drugs which are also palliative and not curative. One should, therefore, be continually searching for new screening methods based on animal models of human disease processes.

Elucidation of the etiology of clinical disease states still requires ex-

tensive effort. When an abnormality in cellular function can be identified as the consequence of a biochemical lesion, then the primary screening method for new drugs will involve a biochemical assay procedure. In the meantime, the pharmacological screening methods of the types described in this volume will be needed for the discovery of new drugs.

Finally, there are no screening methods that do not require the exercise of judgment and discretion on the part of the researcher.

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PETER HEBBORN

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1

α-Adrenergic Blocking Agents

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I. General Considerations

A. Adrenergic Receptors

If receptors may be defined as tissue components with which a drug interacts to produce its characteristic physiological effects, then the adrenergic receptors specifically refer to those components of the effector cells through which the sympathomimetic amines exert their actions. The adrenergic receptors have been further classified into α- and β-receptors on the basis of their relative responsiveness to sympathomimetic amines (Ahlquist, 1948). Although the catecholamines act on both kinds of receptor, some compounds stimulate or block adrenergic responses specifically at either α- or β-receptors; those agents, therefore, can be

divided into α- and β-adrenergic stimulants and α- and β-adrenergic blocking agents.

Blockade at the α-adrenergic receptors can be recognized by comparison of a test substance with the actions of two established sympatholytic agents, now more precisely termed α-adrenergic blocking agents, namely, phentolamine and phenoxybenzamine. The former compound causes a parallel and rightward shift of the agonist (catecholamine) dose–response curve, and the inhibition of response to a dose of an agonist may be reversed by larger doses of the agonist. Phentolamine, thus, is termed a competitive, reversible antagonist. The blocking action of phenoxybenzamine (POB) and other 2-halogenoethylamines has been described by a variety of terms: nonequilibrium antagonism (Nickerson, 1957), insurmountable antagonism (Gaddum, 1957), and competitive, irreversible antagonism (Furchgott, 1955; Kimelberg *et al.*, 1965).

In contrast to phentolamine, phenoxybenzamine does not form a dissociable complex with the receptor. Its binding to the receptor probably involves covalent bond formation and the blockade is prolonged. Experimentally, an effective adrenergic blockade produced by phenoxybenzamine cannot be overcome even by large doses of the agonist. Consequently, in experiments performed *in vitro*, increasing the concentration of phenoxybenzamine results in a progressive depression of response to the agonist until complete abolition of the response is achieved.

The use of pA_x values (Schild, 1947) is a convenient method for evaluating competitive antagonism. pA_x is defined as the negative logarithm of the molar concentration of the antagonist which will reduce the effect of a multiple dose of an agonist to that of a single dose. If the interaction of the drugs at the receptor is bimolecular, then

$$\log (x - 1) = \log K_2 - n\mathrm{pA}_x \tag{1}$$

where x is the ratio of equiactive doses of agonist in the presence and in the absence of antagonist; n and K_2 are constants.

Thus, when $\log (x - 1)$ is plotted against pA_x, a straight line results with a slope equal to $(-n)$, which intersects the pA_x axis at a point corresponding to pA_2 (Fig. 1). When $n = 1$, $pA_2 - pA_{10} = 0.95$, and this difference in pA_2 and pA_{10} values can be used as a test for competitive antagonism, although it is preferable to use a plot of $\log (x - 1)$ over a wide range of antagonist concentrations.

Antagonist activity may be evaluated, also, in terms of the apparent dissociation constant K_B of the receptor–antagonist complex (Furchgott, 1967). The theoretical basis for this procedure is the equation

$$K_B = \frac{B}{x - 1} \tag{2}$$

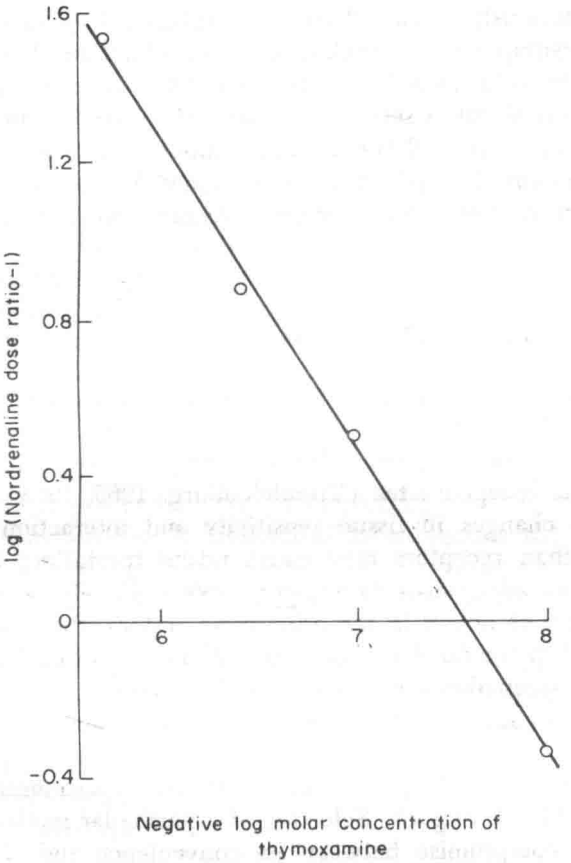

FIG. 1. The antagonistic interaction of thymoxamine with norepinephrine on the guinea pig vas deferens. Thymoxamine was added to the bath 2 min before contractile responses to norepinephrine were obtained. The pA_2 value of 7.57 corresponds to the point of intersection of the regression line with the abscissa. Where the dose ratio equals 0.95, a perpendicular dropped from the regression line to the abscissa gives the pA_{10} value of 6.42. (From Birmingham and Szolcsanyi, 1965.)

where B is the molar concentration of the antagonist and x is the dose ratio of agonist in the presence and in the absence of the antagonist. Under true equilibrium conditions $-\log K_B = pA_2$, as defined by Schild (1947).

An empirical term, pA_h, may be used as a quantitative index of the activity of a compound which reduces the attainable maximum of the dose–response curve for the agonist. pA_h is defined as the negative logarithm of the molar concentration of an antagonist which reduces the maximum response to an agonist to a value which is 50% of the maximum