# RESUME OF LITERATURE.

Drugs which inhibit the responses of autonomic effector cells to adrenergic agents and to sympathetic nerve impulses are of considerable pharmacological and therapeutic interest. For a proper understanding of the pharmacology of these substances, it is essential to know the physiology of sympathetic nerve fibres and the characteristic responses of the effector cells to sympathomimetic agents.

The post ganglionic fibres of the sympathetic nervous system produce their effects by releasing noradrenaline at their terminals. The transmitter released at sympathetic postganglionic nerve endings exerts its effects preponderantly locally at the site of its liberation. However, small quantities may escape into the general circulation and produce minor effects at a distance.

Peripheral excitatory and inhibitory, cardiac, methbolic and central nervous system actions of adrenaline, noradrenaline and other adrenergic drugs are all well known. The relative potencies of various sympathomimetic amines with respect to these actions differ widely. The differences are chiefly of a quantitative nature and it is possible to generalize that all sympathomimetic amines possess some activity in each of these catagories.

Adrenergic stimulation and circulating catecholamines are supposed to be etiological factors in a wide variety of clinical conditions such as benign and malignant hypertension, Burger's disease, Raynaud's disease, thrombophlebitis and many allied conditions ( Victor Drill, 1958; Krantz and Carr, 1961). On the basis of such assumptions a number of the pharmacological agents were introduced so as to reduce, abolish or reverse the effects of the circulating catecholamines either by inhibiting the biosynthesis of catecholamines in the body tissues, or by inhibiting the release of adrenergic transmitters at the postganglionic sympathetic nerve endings or by producing blockade at adrenergic receptors sites.

The adrenergic blockade:

In 1905 Dale published his classical paper " On some physiological actions of Ergot " which contained a remarkably complete and accurate description of the pharmacological activity referred to as adrenergic blockade. This consists of inhibition of many but not all, responses to adrenaline, noradrenaline, other sympathomimetic amines and to sympathetic nerve activity. Stoll (1950) separated different types of alkaloids from ergot and demonstrated the alkaloids responsible for the adrenergic blocking activity. During this period observations were made on several classes of synthetic compounds possessing adrenergic blocking effects. Recently several new types of such agents which are sufficiently specific and nontoxic are introduced for clinical use.

Excitatory responses of vascular smooth muscle and of exocrine gland cell are readily blocked by the classical adrenergic blocking agents and their inhibition is accepted as a criterian for inclusion of agents in the category of adrenergic blocking agents. Reversal, of the pressor response to adrenaline in intact anaesthetized animals is one of the most dramatic expressions of adrenergic blockade and has received major attention but unfortunately critical interpretation of these results is not easy because cardiac, vasoconstrictor and vasodilator components are involved in a variable and unknown relationship to one another. Differences in the ability of adrenergic blocking drugs to reverse the pressor responses to various sympathomimetic amines were reported by Barger and Dale (1910). However, these differences seem to be primarily due to differences in the actions of sympathomimetic amines in question,rather than differences in blocking activity. Inhibitory vasodilator actions of sympathomimetics appeared to be particularly responsible for these differences. Dale's (1906) first study of the adrenergic blockade produced by ergot revealed important differences between the excitation of vascular smooth muscle and that of the cardiac muscle and thus the usual reference to peripheral excitatory effects was limited to those on vascular smooth muscles and exocrine glands, while the cardiac effects were classified separately.

The adrenergic receptor:

These differences in the actions of various sympathomimetic amines led Ahlquist (1948) to propose two separate receptor sites in adrenergic effector cells; these he designed as alpha and beta adrenotropic receptors, the former eliciting excitatory effects while the latter being responsible for inhibitory actions except intestinal relaxation and cardiac stimulation. This classification of adrenotropic receptors suggested by Ahlquist left many areas of adrenergic activity unexplained.

Some recent developments in the classification of receptors for catecholamines will now be discussed. At the 1958 symposium on catecholamines at Bethesda the following modification of Ahlquist's classification of receptors was proposed by Furchgott. Alpha receptors for contraction of smooth muscle, beta receptors for relaxation of smooth muscle other than that of intestine, and also for increases in rate and strength of cardiac contraction; gamma receptors for glycogenolysis and delta receptors for inhibition of intestinal smooth muscle.

The principal basis for separately classifying receptors in the intestine as delta receptors was that the relaxing effect of noradrenaline and adrenaline on isolated segments of intestine could not be effectively antagonized either by dichloroisopropyl-noradrenaline or by alpha-type blocking agents such as dibenamine and phentolamine. However, within a short time after the proposal for delta receptors (Furchgott, 1959) Ahlquist and Levy (1959) found that in the intact dog a combination of dichloroisopropyl-noradrenaline and an alpha-type blocking agent ( namely, Dibozane) could antagonize the inhibitory effects of adrenaline and noradrenaline on intestinal motility. In addition, they found that dichloroisopropyl-noradrenaline alone was able to antagonize the inhibitory effect of isopropylnoradrenaline, and that Dibozane alone was able to antagonize the inhibitory effect of phenylephrine. These investigators therefore concluded that the intestine has both alpha and beta receptors and that activation of either type leads to inhibition of motility. Thus, to antagonize effectively the effects of adrenaline and noradrenaline, which act on both types of receptors one would need the simultaneous presence of antagonists for both alpha and beta receptors. This classification of adrenergic receptors has since been generally accepted.

The alpha or excitatory receptors were found to be most susceptible to the effects of the conventional adrenergic blocking agents. The beta receptors responded to blockade only with massive doses of the adrenergic blocking agents. Powell and Slater (1958) showed that 1-(3', 4'-dichlorophenyl)-2-isoprophylaminoethanol hydrochloride which is a dichloro analogue of isopronaline (DCI) selectively blocked some inhibitory actions of adrenaline and isoprenaline. The cardiac stimulant action of the amines was also effectively blocked by dichloroisopropyl noradrenaline. Recently Nethalide (Alderlin) which is 1-(2-naphthyl)-2-isopropylaminoethanol hydrochloride has also been shown to be effective in blocking the inhibitory and cardiostimulant actions of adrenaline and isoprenaline (Black and Stephenson, 1962).

With the availability of drugs which more or less specifically block adrenergic responses attributed to the beta adrenergic receptor of Ahlquist, adrenergic blocking drugs can now be classified into alpha-adrenergic receptor blocking agents (ergot, dibenamine, phenoxybenzamine, tolazoline, phentolamine, azapetine etc) and beta-adrenergic receptor blocking agents (dichloroisopropylnoradrenaline and Nethalid).

More recently, renewed activity in this field has led to considerable clarification of the loci and the mechanisms of action of various groups of adrenergic blocking agents and the data on adrenergic antagonism has been subjected to various types of quantitative treatment to distinguish between the type of antagonism exhibited by adrenergic blocking agents.

Drug antagonism with special reference to Adrenergic Blocking Agents:

The effects obtained with drugs result from the interactions between the molecules of the drug and the molecules of which the biological object is composed. It may be assumed that activation of a cell by a chemical involves a primary combination of the stimulant with some cell constituent which then triggers off a chain of events or reactions of undetermined length and nature, resulting in the measured response. Antagonists which interfere with the primary combination of the stimulant with some cell constituent, usually exhibit the greatest specificity of action.

There is good evidence that the reaction between the drug molecules and receptors is a bimolecular reaction (Ariens and van Rossum 1957).

$$[R] + [A] \underset{K_2}{\overset{K_1}{\rightleftharpoons}} [RA]$$

Where $[R]$ is the concentration of free receptors, $[RA]$ the concentration of occupied receptors and $[A]$ the concentration of drug A. There is no estimate of the total amount of receptors in a tissue so that we can calculate on the fraction of receptors $[RA] / r$ occupied by the drug.

$$[RA] / r = 1 / (1 + KA / [A]).$$

Where $r$ is the total number of receptors and KA is the dissociation constant of drug A. The affinity of a drug A is defined as the reciprocal of the dissociation constant. The logarithm of the affinity equals the negative logarithm of KA.

As a consequence of receptor occupation by an agonistic drug a stimulus is generated (Ariens, van Rossum and Koopman, 1960; van Rossum 1958; Stephenson 1956).

$$S_A \ / \ S_m = \alpha \cdot [RA] / r$$

Where $S_A / S_m$ is the numerical value for the stimulus generated by a certain dose of drug A, while $S_m$ is the maximum possible stimulus to be obtained by the particular tissue. The proportionality constant, $\alpha$, has been called the "instrinsic activity" (Ariens 1954; Ariens, van Rossum and Simonis, 1956).

The intrinsic activity determines whether the drug can generate a stimulus and therefore whether a drug can be "active" or not. The affinity and the intrinsic activity are determined by the molecular properties of both receptor and drug.

Competitive antagonism:

The result of the combining of the compounds will depend on the relationship between affinities and intrinsic activities of the compounds. Burgi's law indicates that in a homologous series the antagonistic character increases with decreasing intrinsic activity assuming the affinity to remain fairly constant; and a compound with zero intrinsic activity is in fact a competitive antagonist (Veldstra, 1956). Competition for the receptor will usually decrease the frequency of the best interaction and with decreasing intrinsic activity of one of the components the combined action will more and more take the form of a competitive antagonism.

Most compounds acting in this fashion appear to be in mass action equilibrium with the receptors and the blockade produced is a measure of competition between the agonist and antagonist for receptor occupancy. Such agents are referred as classical competitive antagonists.

Recent studies have demonstrated that certain blocking agents react with the receptor or some adjacent group to form a relatively stable chemical bond. This reaction precludes further mass action "competition" and effectively reduces the number of available receptors (Nickerson, 1956). Such agents are best described as non-equilibrium antagonists e.g. organophosphorus anticholinestrase agents (DFP, TEPP), beta-halo alkylamine adrenergic blocking agents ( phenoxybenzamine and its congeners ). The blockade produced has been referred to also as "Irreversible competitive" (Furchgott, 1955) and "unsurmountable" ( Gaddum et al., 1955). However, the blockade is not strictly irrversible and the term unsurmountable is appropriate only when the antagonist is used in sufficiently large doses to prevent a maximal response even in presence of massive amount of agonist.

Tests for competitive antagonism:

(1) Clark and Raventos (1937) suggested a method of estimating the activity of drug antagonists in terms of "the concentration which altered by a selected proportion e.g. 10 fold the concentration of an active drug needed to produce a selected effect. The negative logarithm of this (molar) concentration has been termed pAx where x is the proportion selected (Schild 1947).

The pAx is a measure which is particularly suitable for determining the activity of antagonists which do not alter the slope of the log dose effect curve of the agonist. Difficulties arise, however, with the antagonists which affect this slope, pAx values may then be determined when the effect of the agonist is half the maximal (Schild 1949).

One of the two tests for competitive antagonism is based on a comparison of equiactive doses. This type of test involves no assumption about the manner in which the receptors and physiological effect are related. It assumes only that equal effect involve equal number of receptors. There are two stages to the test. First a series of log-dose-effect curves are plotted, one without antagonist and the others with different concentrations of the antagonist. If these curves are parallel, they afford presumptive evidence of competitive antagonism (Arunlakshana and Schild 1957). Next the equation given by Gaddum for this case may be written as follows:

$$\frac{y}{1-y} = K_1 A = \frac{K_1 Ax}{K_2 B^n + 1}$$

(where $y$ is the fraction of the active receptors)

$K_1$, $K_2$, $n$ are constants.

$A$ and $Ax$ are the concentration of agonist causing the response corresponding to $y$ in the absence and presence respectively of the antagonist in concentration B.)

Eliminating $K_1 A$ and taking logarithm

$$\log (x-1) = n \log B + \log K_2$$

Since $pAx = -\log B$.

$$\therefore \log (x-1) = \log K_2 - n\, pA_2$$

The plot of log (x-1) against pAx (-log B) thus gives a straight line with slope -n. The line intersects the pAx axis at a point corresponding to pAx.

When $n = 1$ $pA_2 = \log K_2$

and $pA_2 - pA_{10} = 0.95$

This equation provides a useful test of competitive antagonism which is independant of y . This relation is readily verified experimentally. However, (i) Depression by a maximal dose of agonist, (ii) a change in receptor / effect relation by gradual alteration in sensitivity to the agonist and (iii) failure of the antagonist to reach equilibrium are recurrent sources of error and may invalidate the results.

To add to the significance and reliability of the results, testing of competitive antagonist should be explored over a wide range of concentrations.

(2) If a competitive antagonist produces the same pAx in different biological test preparations it can be assumed that it reacts with closely similar receptors. If this assumption holds true then it can be deduced that if the different preparations with closely similar receptors produce the same pAx value then it is competitive antagonism. Agonists are as a rule more variable than antagonist e.g. the guineapig ileum is much more sensitive to histamine than the tracheal chain but the pAx values of antagonists in the two preparations are the same (Arunlakshana and Schild 1957). Stephenson and Ariens (1953-54) have suggested that the activity of an agonist depends on at least two factors; its affinity for the receptor and the contribution made by the drug receptor complex to the physiological effect, whereas the activity of a competitive antagonist depends

only on its affinity for the receptors.

(3) Receptor protection:

If competitive blockade involves the occupation of the specific agonist receptors by the antagonist, the presence of the agonist in high concentrations will project the specific receptors from the blockade and this can be verified after removal of both the agonist and the unreacted antagonist. The specificity of such " receptor protection" tests is indicated by the fact that where an antagonist is effective against responses to several types of stimulants such as adrenergic, cholinergic, histamine etc. the presence of agonist during exposure to the blocking agent inhibits the blockade of responses to only one specific type of agonist (Furchgott 1954).

<u>Differentiation between classical competitive and non-equilibrium (irreversible competitive) antagonists</u>:

No single clear cut test is available to differentiate classical competitive from non-equilibrium blockade of tissue responses to adrenaline and noradrenaline. However, certain tests may be suggestive of the type of antagonism.

(1) Straight-line relationship in plots of agonist versus antagonist is characteristic of classical competitive agent, whereas lines describing the action of nonequilibrium agents curve toward the agonist axis (Nickerson, 1957).

(2) Development of the blockade produced by classical competitive agent ceases and is reversed as soon as active drug is removed from the surrounding medium (biophase) whereas formation of stable drug receptor complex from active nonequilibrium inhibitor present in biophase may continue for sometime after washing. It may be mentioned that some classical competitive antagonists such as atropine and ergotamine produce relatively prolonged blockade. Nachmansohn et al., (1947) and Nickerson et al., (1949) have demonstrated that the reactions of all known non-equilibrium inhibitors with receptors appear to occur in two steps, the first reversible "adsorption" and the second a more stable chemical reaction. From this it can be concluded that rate of dissipation of classical competitive agents when washed out of tissues is a continuous function of the concentration gradient whereas the dissipation of non-equilibrium blocking agents reveals a discontinuity.

(3) The cumulative effect of several short exposures to a competitive blocking agent should be less than that produced by a single exposure of equal total duration whereas cumulative effect of short exposures to a non-equilibrium agent should be greater than the effect of the single longer treatment although experimental verification of this assumption has been limited to a relatively small number of compounds. Comparison of the effects of single and repeated exposures has provided a clear differentiation of blockade produced by dibenzyline

from that due to several competitive inhibitors (Nickerson 1957 ).

Non-competitive antagonism:

A second type of blockade produced by agents which act at some point between the receptors and the ultimate response is designated as non-competitive antagonism. Non-competitive antagonists have received considerably less attention than competitive.and non-equilibrium agents largely because they tend to exhibit much less specificity of action.