RECEPTORS FOR ERGOT ALKALOIDS

IN

GUINEA-PIG ILEUM AND DOG URINARY BLADDER

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Maqsud Ahmad Cheema

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ABSTRACT

Ergot alkaloids cause most smooth muscles to contract. It is known that this action is due to combination with α-adrenergic receptors in smooth muscles where noradrenaline is excitatory, e.g. rabbit aorta and uterus, dog retractor penis, cat nictitating membrane. There are many smooth muscles, however, where noradrenaline causes relaxation and yet ergot alkaloids are excitatory. The receptors on which ergot alkaloids act to produce their effect on tissues with these properties have therefore been studied. Two in vitro preparations were used, strips of dog urinary bladder which relaxed in response to noradrenaline, and segments of guinea-pig ileum taken from a region in which both α- and β-adrenergic receptors subserve inhibition. The results indicate that the ergot alkaloids cause contraction of these preparations by acting on 5-hydroxytryptamine receptors.

Four ergot alkaloids were tested, dihydroergotamine, ergotamine, ergonovine, and methylergonovine. Ergotamine and dihydroergotamine did not cause guinea-pig ileum to contract, but inhibited the contractile action of 5-hydroxytryptamine. Ergonovine and methylergonovine behaved as would be expected of partial agonists on 5-hydroxytryptamine receptors, in that the maximal contraction they could induce was smaller than that of 5-hydroxytryptamine, and that they antagonized the action

of 5-hydroxytryptamine. In dog urinary bladder all four ergot alkaloids caused contraction. Ergonovine and methylergonovine were more potent than 5-hydroxytryptamine, and were used for most of the studies since contractions due to ergotamine and dihydroergotamine were unduly prolonged.

Large doses of 5-hydroxytryptamine are known to desensitize the guinea-pig ileum selectively to subsequent doses of 5-hydroxytryptamine. This procedure also inhibited the action of the ergot alkaloids, without inhibiting the actions of acetylcholine or histamine, which act on other receptors. Similarly, large doses of ergonovine or methylergonovine desensitized the ileum to 5-hydroxytryptamine without affecting the responses to other types of agonist.

Dose-ratios were compared for the ergot alkaloids, 5-hydroxytryptamine and several other agonists in guinea-pig ileum preparations exposed to various antagonists and inhibitory procedures. The results of these experiments gave further evidence for an action on 5-hydroxytryptamine receptors, but were more difficult to interpret, since two types of 5-hydroxytryptamine receptor are believed to exist in the guinea-pig ileum, M receptors in the intramural nerve plexuses, and D receptors, probably in the smooth muscle cell. M receptor blocking agents, morphine, atropine and cocaine, markedly reduced responses to 5-hydroxytryptamine but had much less effect on the ergot alkaloids. Similarly, prolonged cold storage, presumed to inactivate the intramural plexuses, or the ganglionic depolarizing agent dimethylphenylpiperazinium depressed the action of 5-hydroxytryptamine more than that of the ergot alkaloids. However, the effects of large doses of the ergot alkaloids were markedly depressed by cold storage. 2-Bromolysergic acid diethyl-

amide and phenoxybenzamine, D receptor blocking agents, antagonized the ergot alkaloids much more than did the M receptor blocking agents. In general, the results were such as might be expected if the major action of the ergot alkaloids were on D receptors.

In the dog urinary bladder doses of morphine or atropine which blocked M receptors elsewhere did not inhibit the ergot alkaloids or 5-hydroxytryptamine. 2-Bromolysergic acid diethylamide inhibited the ergot alkaloids and 5-hydroxytryptamine to an equal degree. pA_2 values for its antagonism of ergonovine, methylergonovine and 5-hydroxytryptamine were almost equal, and differed greatly from its effect on other types of agonist, acetylcholine and histamine. All four ergot alkaloids were potent antagonists of 5-hydroxytryptamine. It was concluded that the ergot alkaloids act on 5-hydroxytryptamine receptors of dog urinary bladder also, and that this tissue has no M receptors.

To

My Wife

Naseem

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A. INTRODUCTION

Ergot, known botanically as Claviceps purpurea, is a parasitic fungus which grows on rye, so that some of the grains become replaced by dark poisonous growths similar in shape to normal grains but larger. It grows in China, Russia, France and Spain and is favoured by moist, warm weather.

Ergot has a remarkable history. Man first made contact with it as a poison when the consumption of rye infected with ergot led to severe symptoms of ergotism. Ergot has been responsible for human misery through the centuries. During the Middle Ages, epidemics of ergot poisoning, called Holy Fire or St. Anthony's Fire, were frequent, particularly after a warm, wet growing season. People would suffer from agonizing burning sensations in the extremities which would, later on, become gangrenous, and drop off without loss of blood. Many pregnant women would abort and consequently die. The parasitic nature of ergot was not appreciated until towards the end of the eighteenth century; earlier it was looked upon merely as diseased rye. With the recognition of the cause of the disease, outbreaks of ergotism have become less and less frequent, although it has occurred as late as 1953 in France (Goodman and Gilman, 1955).

Ergot was known as obstetrical herb before it was identified as the cause of St. Anthony's Fire. How this poison came to be used as a remedy is not quite clear. The fact that ergot has a powerful stimulant action on the uterus and can be used to hasten childbirth was known to German midwives in the sixteenth century but was kept a secret as long as possible (Gaddum, 1959). It was not until 1808 that John Stearns

described the oxytocic action of ergot. He recommended its use to induce and hasten labour. Its general acceptance greatly increased the number of still-births so that Hosack in 1824 described it as "pulvis ad mortem" rather than "pulvis ad partum". He recommended that ergot should only be used to control post-partum haemorrhage, which is its only accepted use in the practice of obstetrics today.

B. THE CONSTITUENTS OF ERGOT

Ergot has been called a "veritable treasure house of pharmacological constituents" (Goodman and Gilman, 1955). Barger (1931) divided the substances isolated from ergot into two main groups. In the first group are those products peculiar to ergot and not obtainable from any other source, the important members being the ergot alkaloids. Certain pigments peculiar to ergot but of no known pharmacological significance are also found. The second group consists of a larger number of compounds normally obtainable from other sources. This heterogenous group includes inorganic constituents, carbohydrates, glycosides, sterols, amino acids, amines, and quaternary ammonium bases. Of these, acetylcholine, choline, histamine, and tyramine are of pharmacological importance.

C. CHEMISTRY OF ERGOT ALKALOIDS

Ergot has been the object of intensive chemical investigations for many years, mainly by Jacobs and his associates as well as Stoll and his colleagues. This work has been reviewed by Stoll (1952), Glenn (1954), Saxton (1956) and Kornfeld (1958). It is now believed that the

entire pharmacological activity of crude ergot can be accounted for by the chemically identified products obtainable from it.

The history of the chemistry of the ergot alkaloids is confusing because preparations once considered to be pure alkaloids are now known to be mixtures of several substances. The first isolation of a crystalline, pharmacologically active substance from ergot was accomplished in 1906 by Barger, Carr and Dale and independently by Kraft. This was named ergotoxine and was thought to be a pure alkaloid. It is now known to be a mixture. In 1920, Stoll isolated ergotamine which ultimately proved to be the first pure ergot alkaloid. Neither of these compounds, however, produced the therapeutic effects of extracts of crude drug (Moir, 1932). In 1935, almost simultaneously four different research teams announced the isolation of a new alkaloid. In Britain, Dudley and Moir named their compound ergometrine. In Switzerland, ergobasine was isolated by Stoll and Burckhardt; while in America, ergostetrine was described by Thompson and ergotocine by Kharasch and Legault. It was subsequently shown that the four compounds were identical in chemical structure and pharmacological properties (Chen, Swanson, Kleiderer and Clowes, 1936), and the name ergonovine was assigned to the alkaloid. Stoll and Hofmann (1943) demonstrated that ergotoxine was not a pure compound but a mixture of three alkaloids, ergocristine, ergocornine and ergokryptine.

Ergot alkaloids occur in readily interconvertible, stereoisomeric pairs. For convenience the alkaloids have been named with ending of "ine" for levorotatory isomers and "inine" for dextrorotatory isomers. Only the levorotatory isomers, which are derivatives of lysergic acid (an indole compound), have pharmacological importance. Very little action

is possessed by the dextrorotatory isomers, which are derivatives of iso-lysergic acid (Stoll, 1952; Glenn, 1954). The spatial changes in the molecules, which result from the interchange of the H for the COOH at C-8 in the lysergic acid portion of the alkaloid, markedly reduce the activity (Stoll, 1950).

Chemically, the natural ergot alkaloids are of two types:

(i) Amino acid alkaloids: This group includes ergotamine, ergocornine, ergokryptine, and ergocristine. These are polypeptide derivatives of lysergic acid and have higher molecular weights. Upon hydrolysis, these alkaloids yield ammonia, d-proline, lysergic acid, one other amino acid, and either pyruvic or dimethylpyruvic acid.

(ii) Amine alkaloid: Ergonovine is the only known naturally occurring alkaloid in this group. From a chemical stand point, ergonovine is the simplest compound (Fig. 1). Upon hydrolysis, it yields lysergic acid and d-2-amino-propanol.

The variety of the ergot alkaloids has been further extended by two types of semisynthetic derivatives:

(i) The double bond between C-9 and C-10 of lysergic acid can be selectively saturated; in this manner a well-defined series of stable dihydrogenated alkaloids has been obtained (Stoll and Hofmann, 1943). These have been named as dihydroergotamine, dihydrocristine etc. They possess somewhat different pharmacological properties than the parent alkaloids; their is loss of oxytocic activity but increased adrenergic blocking activity.

(ii) Lysergic acid has been combined with amines differing from those linked to it in nature. Lysergic acid-diethylamide (LSD) and lysergic acid-hydroxybutylamide (Methylergonovine) are examples of this group.

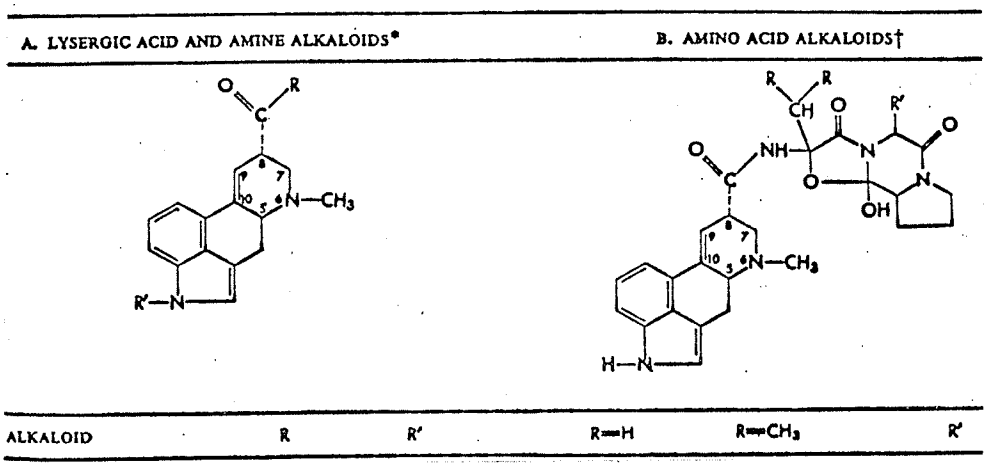

Fig. 1.

Ergonovine	$-NH-\underset{\displaystyle CH_3}{\underset{\vert}{C}}HCH_2OH$	H	Ergotamine	$-CH_2-$ 
Methylergonovine	$-NH-\underset{\displaystyle CH_2CH_3}{\underset{\vert}{C}}HCH_2OH$	H	Dihydroergotamine differs only in being saturated at C_9 and C_{10}	

During recent years, research work regarding the synthesis of various lysergic acid derivatives has been extremely fruitful. Apart from synthesis of lysergic acid, LSD, ergonovine, methylergonovine and many other analogues, Hofmann and his colleagues have been successful in the synthesis of ergotamine. A large number of its analogues are expected to be synthesised and this is bound to open a new era in the pharmacology of ergot alkaloids (Rothlin, 1964).

D. PHARMACOLOGICAL INVESTIGATIONS OF ERGOT ALKALOIDS

The pharmacology of the ergot alkaloids is highly complex; their pharmacological actions are varied and unrelated. The major actions are stimulation of smooth muscle especially vascular and uterine, adrenergic blocking action, and complex excitatory and depressant effects on the central nervous system. These actions are present to a varying degree in different alkaloids. Antagonism of 5-hydroxytryptamine (5-HT) by some of these compounds has been responsible for continued interest in them. Only the literature related to the problem under study will be reviewed in some detail and therefore no mention will be made about the actions of ergot compounds on the central nervous system.

Early Studies. Although experimental work about ergot has been reported earlier, the pharmacology of ergot virtually starts with the fundamental work of Dale (1906). Since then the ergot alkaloids have been extensively investigated, but few basic observations have been made which were not indicated by the classical studies of Dale. Rothlin and his colleagues, among others, have contributed greatly to the later studies of ergot compounds, especially those of dihydrogenated derivatives.

Early work on ergot alkaloids was excellently reviewed by Barger (1931, 1938). Nickerson (1949, 1959) reviewed the vast amount of literature concerning pharmacological studies of ergot alkaloids during later years, critically analysed various conflicting reports, and pointed out the various pitfalls and limitations of experimental work done in connection with ergot alkaloids. He pointed out the danger of placing too much emphasis on the adrenergic blocking activity of ergot compounds and overlooking their other important pharmacological properties with the consequent misinterpretation of experimental results. He stressed that other pharmacological actions, such as smooth muscle contraction and complex effects on the central nervous system, are produced by doses smaller than those required for adrenergic blockade.

Actions on Smooth Muscle. One of the most characteristic effects of the ergot alkaloids is a direct stimulation of smooth muscle in many organs (Dale, 1906). This effect was known long before the demonstration of adrenergic blockade by these agents (Barger, 1931, 1938). In the case of ergot alkaloids lacking a peptide substituent e.g. ergonovine, uterine stimulation is prominent in the complete absence of adrenergic blocking activity (Brown & Dale, 1935). The dihydrogenated derivatives show a marked reduction in smooth muscle stimulation. Rothlin (1944, 1946, 1947) has shown that dihydrogenated compounds not only fail to cause contraction of rabbit or guinea-pig uteri in vitro but also tend to diminish uterine tone and activity and to inhibit the excitatory effects of ergotamine and ergonovine on the uterus.

All the natural ergot alkaloids cause peripheral vasoconstriction due to direct action on vascular smooth muscle. Isolated arteries and perfused blood vessels respond to these drugs by constriction. In

the pithed cat, blood pressure rises without any significant change in heart rate. Ergotamine is the most potent vasoconstrictor. Hydrogenation of the alkaloids reduces the action on vascular smooth muscle. It is not abolished completely as demonstrated by a pressor response in pithed cats or in patients with spinal cord transection (Rothlin, 1947; Freis, Stanton, Litter, Culbertson, Halperin, Moister and Wilkins, 1949). It is now well established that ergotamine and probably other natural ergot alkaloids are potent coronary vasoconstrictors, but the hydrogenated alkaloids are much less active (Katz and Lindner, 1939; Scherf, Perlman and Schlachman, 1949). Cyanosis and gangrene in the rat's tail or cock's comb is a prominent response to natural ergot alkaloids. Production of cyanosis in the cock's comb was for many years the official assay method for extract of ergot (USP XII). The early development of transient cyanosis is probably largely due to a vasoconstrictor action, but other factors are probably involved in the later development of "thromboangitis" and gangrene (Yater and Cahill, 1936; Loewe and Lenke, 1938). The hydrogenated alkaloids appear to have much less tendency to produce gangrene (Orth and Ritchie, 1947).

Both ergotamine and ergotoxine produce miosis in the cat. This effect is largely the result of direct stimulation of the sphincter muscle of the iris (Yonkman, 1931). In rodents, the ergot alkaloids, including ergonovine, produce mydriasis rather on miosis in both the normal and sympathetically denervated eye; this response is the result of direct stimulation of the radial muscle of the iris (Barger and Dale, 1907). The species difference in response of the iris to ergot alkaloids may be due to differences in the relative strengths of the dilator and constrictor muscles.

Ergot alkaloids have been shown to cause an increase of peristalsis throughout the gastrointestinal tract in anesthetized and unanesthetized animals. the exact mechanism is not understood. Brown & Dale (1935) showed that ergonovine produced a weak but quite definite inhibitory response in rabbit isolated jejunum. This effect was abolished by ergotoxine. They suggested that this action of ergonovine has a sympathomimetic component. In contrast, they found that ergonovine produced a tonic contraction of guinea-pig isolated jejunum. This action of ergonovine was also readily antagonized by ergotoxine. The possibility of this action of ergonovine being sympathomimetic was ruled out, as the action of adrenaline on this preparation is purely inhibitory. Ergotoxine had no stimulatory action of its own on either tissue.

Adrenergic Blocking Action. The first demonstrations of specific adrenergic blockade were those of Dale (1905) and Sollman & Brown (1905) who used crude or only partially purified ergot preparations. In spite of extensive investigations over several decades, the results achieved through the classical studies of Dale (1906) still remain representative of the action of purified ergot alkaloids containing a polypeptide side chain.

All members of the ergotoxine complex are more potent adrenergic blocking agents than ergotamine. Hydrogenation considerably increases the potency of all these alkaloids. Both the natural and dihydrogenated alkaloids produce a blockade of rather short duration. These drugs are relatively easily 'washed out' of in vitro smooth muscle preparations. Their blocking effect disappears before their other effects.

Early experiments of Dale (1906, 1913) showed that large doses of the ergot alkaloids produce a blockade which is effective against in-

jected adrenaline and strong adrenergic stimuli. This property is shared by their dihydrogenated derivatives (Nickerson, 1949). Ergot compounds produce a more effective blockade than that produced by any of the other adrenergic blocking agents except β-haloalkylamines. Considerably larger doses of ergotamine and ergotoxine are required to inhibit the pressor response to splanchnic nerve stimulation than that to injected adrenaline (Dale, 1906; Barry, 1937).

The natural and dihydrogenated ergot alkaloids do not affect adrenergic chronotropic and inotropic cardiac responses in mammals, even in massive doses (Dale, 1906; Rothlin, 1944, 1945, 1947). It has been repeatedly reported that ergot alkaloids produce a marked cardiac slowing. This is not due to adrenergic blockade but to central vagal stimulation; bradycardia persists after sympathectomy but not after vagotomy (Moore and Cannon, 1930; Rosenblueth and Cannon, 1933).

Both the natural and dihydrogenated ergot alkaloids antagonize the inhibitory response of isolated rabbit intestine to adrenaline and noradrenaline (Rothlin, 1929; Nanda, 1931, Rothlin et al., 1954). The specificity of the blockade has been questioned (Nickerson, 1949, 1959). Rothlin (1954), however, has shown that this antagonism is not abolished by hexamethonium or atropine in doses that suppress nicotine and acetylcholine responses indicating that the influence of the alkaloid is not due to the excitation of cholinergic mechanisms. The antagonism also occurs with concentrations of the alkaloids which do not significantly alter the relaxation induced by papaverine. Recent work has established that there are both α and β-adrenergic receptors in the small intestine of dog (Ahlquist and Levy, 1959), rabbit (Furchgott, 1960) and guinea-pig (Kosterlitz and Watt, 1964; Wilson, 1964). Both these receptors subserve

inhibition. A complete blockade of inhibitory responses to adrenaline, which activates both α and β-adrenergic receptors, by α receptor blocking agents such as ergot alkaloids, does not seem to fit into the present concept of adrenergic mechanisms. It is possible that this blockade by ergot alkaloids is either unspecific or is due to the fact that the ergot alkaloids may also block β-receptive mechanism (Youmans, Green and Denison, 1955).

Similarly, no conclusive demonstration of specific blockade of inhibitory responses of vascular and uterine smooth muscle to adrenaline and noradrenaline by either the natural or dihydrogenated ergot alkaloids has been made.

The natural as well as dihydrogenated alkaloids inhibit adrenaline-induced hyperglycemia more effectively than other α-adrenergic blocking agents. There is no correlation between the ability of various ergot alkaloids to prevent adrenaline hyperglycemia and to block vascular and other smooth muscle responses to adrenaline (Harvey, Wang and Nickerson, 1952).

Ergot Alkaloids as Antagonists of 5-Hydroxytryptamine. As the present study is mainly concerned with the identification of excitatory receptors for ergot alkaloids in tissues which catecholamines inhibit but where 5-HT is excitatory the literature regarding the interactions of ergot alkaloids and 5-HT in various smooth muscles is reviewed in some detail.

Ergot derivatives were among the first compounds used as antagonists of 5-HT. The antagonism of ergotoxine and tryptamine, which forms part of the molecule of lysergic acid, was described by Laidlaw (1911) and that of ergotamine and α-methyltryptamine by Seki (1929). Heymans,

Bouckaert, and Moraes (1932) found that ergotamine antagonized the vasoconstrictor action of defibrinated blood, which was presumably due mainly to 5-HT. Gaddum, Pert, and Vogt (1949) obtained similar results with dihydroergotamine.

Gaddum and Hameed (1954) studied the antagonism of ergot alkaloids and 5-HT on rat uterus, rabbit ear, and guinea-pig ileum. Various derivatives of lysergic acid were compared for their effects on the rat uterus as antagonists of 5-HT and placed in the following descending order of potency: lysergic acid diethylamide, dihydroergotamine, dihydroergocornine, and dihydroergokryptine. Ergotamine, ergotoxine, and ergonovine generally caused great spontaneous activity in the concentrations used, but in all cases there was some evidence that they reduced the response to 5-HT without reducing the response to a choline ester. They also compared the effects of some of these compounds on the rabbit ear, on the vasoconstrictor actions of 5-HT and adrenaline. LSD specifically abolished the constrictor effect of 5-HT without affecting constrictor effects of equi-effective doses of adrenaline. Dihydroergotamine was also more active as an antagonist to 5-HT, but its action was not as specific as that of LSD. Ergotamine was less effective in antagonizing 5-HT and ergonovine ineffective. The antagonism between 5-HT and the ergot alkaloids on the guinea-pig ileum was comparatively feeble and less clearly specific. Low concentrations of LSD diminished the response of guinea-pig ileum to 5-HT by half. Low concentrations of dihydroergotamine had no effect but higher concentrations reduced responses to 5-HT and histamine equally well.

The activity of lysergic acid diethylamide, a potent antagonist to 5-HT on many isolated organs, is due to the d-isomer; the l-isomer

is inactive. Because of the fairly high specificity of LSD on the rat uterus, it has been used extensively by many investigators as a reference standard for evaluating the relative potency of other antagonists of 5-HT (Cerletti and Konzett, 1956; Savini, 1956; Cerletti and Doepfner, 1958). In contrast to Gaddum's earlier observations, Cerletti and Doepfner (1958) found that ergonovine and its derivatives have marked antagonistic activity against 5-HT in rat uterus. Whereas ergonovine and dihydroergonovine exhibited considerable blocking activity, methylergonovine and dihydromethylergonovine approached the potency of LSD.

Rothlin (1957) and Gyermek (1961) have reviewed the actions of a large number of lysergic acid derivatives, which have been synthesized and investigated during the last decade. Some of these surpass LSD in anti-5 HT potency. The best known 2-Bromolysergic acid diethylamide (BOL 148), which is approximately as potent as LSD on isolated organ preparations (Sollero, Page and Salmoiraghi, 1956; Cerletti and Doepfner, 1958; Vane, 1959). Unlike LSD, BOL has less tendency to stimulate the uterus and has no direct constrictor action of its own on blood vessels when tested on rabbit ear (Savini, 1956). Another derivative of LSD, 1-methyl-lysergic acid butanolamide has been found to be four times more active than LSD on rat uterus (Fanchamps, Doepfner, Weidman and Cerletti, 1960). This compound is very active in vivo and is reported to be of value in the symptomatic treatment of patients suffering from malignant carcinoid syndrome (Robson and Stacey, 1962).

Recent Studies. During recent years, quite significant developments have further elucidated the mechanisms of action of ergot alkaloids. Konzett (1960) showed that Dibenamine and phentolamine in doses which do not block 5-HT antagonized the effects of ergometrine on the blood pres-

sure and in vivo contraction of the uterus of the rabbit, thus presenting indirect evidence for the sympathomimetic action of ergonovine on the uterus. Recently, in accordance with the results of Konzett (1960), Fregnan and Glässer (1964) have shown that adrenergic blocking agents, such as phenoxybenzamine, piperoxan and Hydergine, given intravenously in doses sufficient to fully antagonize the responses to adrenaline and noradrenaline on the uterus and blood pressure of rabbit, inhibited the effect of ergonovine without affecting the responses to oxytocin.

Innes (1962a) has presented clear-cut direct evidence for the identification of the smooth muscle excitatory receptors for ergot alkaloids. He used the selective receptor protection technique of Furchgott (1954) to distinguish receptors for ergotamine and ergonovine. On rabbit aortic strips, dog retractor penis, and rabbit uterus, receptors for adrenaline were selectively protected by high concentrations of adrenaline throughout exposure of the preparation to a blocking concentration of ergotamine or phenoxybenzamine. Protected muscles responded to ergotamine; unprotected muscles did not. Muscles where receptors for acetylcholine, histamine or 5-HT were protected by high concentrations of these drugs did not respond to ergotamine. Ergonovine, which has no blocking action on adrenaline receptors, behaved in the same way as ergotamine; muscles which were protected by adrenaline against blockade by phenoxybenzamine responded to ergonovine, but unprotected muscles did not. The stimulant actions of adrenaline, ergotamine, and ergonovine were also protected against the blocking action of phenoxybenzamine by treating the muscle with a high concentration of ergonovine instead of adrenaline. It was concluded that, in smooth muscle where adrenaline is stimulatory, ergotamine and ergonovine act by combining with adrenaline receptors and,

moreover, ergotamine could be regarded not only as an adrenaline antagonist but also as a partial agonist due to its ability to excite the same receptors.

Thus Innes has clearly shown that ergot alkaloids act on adrenergic α receptors in smooth muscles where the action of adrenaline is stimulatory. However, there has been no report regarding any clear evidence for extending this view to smooth muscles whose activity is inhibited by adrenaline. Upon which specific receptors do ergot alkaloids act in such smooth muscles? The present study is designed to answer this question.

CHAPTER 2

DIFFERENTIATION OF SMOOTH MUSCLE RECEPTORS

A. RECEPTOR CONCEPT

B. METHODS FOR DIFFERENTIATION OF SPECIFIC DRUG RECEPTORS

Differentiation by Antagonists

Differentiation by Agonists

Specific Desensitization

Receptor Protection

As the present study is mainly concerned with the differentiation of specific receptors for a particular group of drugs, it seems appropriate to give a brief description of the general concept of drug receptors and the methods generally used in the differentiation of specific receptors.

A. RECEPTOR CONCEPT

The effect of a given drug on a given effector system, such as activation of smooth muscle with consequent contraction or relaxation, is usually interpreted in terms of an interaction between the drug and a specific receptive substance or receptor of the cells of the effector system.

The notion of a specific receptive substance as a site of action for drugs, such as nicotine and curare in the myoneural junction, was introduced by Langley (1905). Since then the term receptor has been indispensable in reasonings on the basis of drug action.

It is not possible to give an exact definition of the term receptor; because neither has the receptor been identified as an individual chemical entity nor has it been possible to study the primary chemical or physical change which occurs when the drug and the receptor interact. The term receptor, therefore, has different connotations and there is no unanimity of opinion, even amongst pharmacologists.

In the present study, the term receptor will be used to indicate the postulated specific molecular sites or structures in or on an effector cell with which molecules of a specific agonist must react in order to elicit the characteristic response of the cell to agonist and still exclude the various types of binding sites included under the

general term drug "Acceptor" (Fastier, 1964).

Current theories of the mechanism of action of drugs at the receptor level rest primarily on the classical work of Clark (1926,1933, 1937) and Gaddum (1926, 1937) who showed that drug receptor interactions closely approximate the relationships encompassed by the Langmuir (1918) adsorption isotherm, which is similar to the Michaelis-Menten equation. Based on these studies, Clark (1937) proposed the equilibrium theory, according to which the drug receptor interactions are governed by the laws of mass action. In its simplest form Clark's theory involves the assumption that the effect is proportional to the number of receptors occupied by the drug, i.e., the maximal response occurs when all receptors are occupied. In order to explain competitive antagonism, Clark hypothesized that both the agonist and the antagonist compete for the same receptors and the effect is directly proportional to the amount of agonist which is combined with the receptors.

Recent work has revealed several facts which cannot be explained by this theory in its simplest form and has thrown doubts on Clark's assumption regarding proportionality between effect and receptor occupancy. Some of these facts are as follows: (a) the slope of the log-dose-effect curve is often significantly different from that predicted by the theory; (b) some of the compounds in a series of analogues are weak agonists by themselves and yet antagonize the effects of a strong agonist. Quantitative studies with such compounds and consideration of the above mentioned anomalies led to the concept of intrinsic activity (Ariens, 1954) or efficacy (Stephenson, 1956). It was pointed out by Stephenson (1953, 1956) that the effect of an agonist depends not only on its affinity for the receptors, but also on its ability to produce an

effect when combined. This idea was developed independently by Ariens (1954) who spoke of the affinity and the intrinsic activity of the drugs. According to this modification of Clark's theory, a drug may antagonize other drugs by occupying nearly all the receptors and yet produce a small effect itself -- a 'partial agonist' or a drug with 'dualism in action'.

Paton (1961) introduced an elegant theory of the mechanism of drug action in which he has shown a complete departure from the basic concept of Clark's classical theory as well as its modifications by Ariens and Stephenson. It is based on the idea that drugs act on receptors only at the moment of combination. He suggested that many of the phenomena of drug action such as dualism in action, competitive antagonism and self desensitization, etc. can be interpreted on the assumption that the stimulant effect produced by a drug depends, not on the number of receptors occupied, but on their rate of occupation. He has thus assigned a crucial role to the dissociation constant of a drug. He provided evidence to show that it is this property of drugs which determines their agonistic, dualistic and antagonistic activity (Paton, 1961; Paton and Waud, 1962, 1964).

Recently, Mackay (1963) suggested that actions of drugs may be explained by assuming that the net flux of a drug across the cell membrane causes depolarization leading thereby to effect. This idea is reminiscent of Straub's potential theory (Straub, 1907) which stated, basically, that drugs produce effects so long as there is a concentration difference. Mackay proposed a 'flux-carrier' hypothesis, in which the net rate of flux of the cationic agonist through the cell membrane into the cell would determine the extent of depolarization and in which the agonist passes through the membrane in association with a 'carrier'. He has

shown how such a hypothesis could be applied to the problems of partial agonistic action, antagonism and desensitization.

More recently, Belleau (1964) has pointed out that the Ariens-Stephenson modifications of Clark's classical theory as well as the rate theory of Paton suffer from the absence of a biophysical basis and do not provide a qualitative interpretation of drug properties at the receptor level. Consequently he has advanced a molecular theory, called Macromolecular Perturbation Theory. He has elaborated this theory for the muscarinic cholinergic receptors and has suggested that the muscarine receptor is acetylated acetylcholinesterase.

The various receptor theories proposed from time to time have been found to be inadequate to fully explain the mechanism of all types of drug action. It is evident that these concepts will be modified and expanded as more and more observations are made in the field and the exact nature of receptor is revealed. At present, any attempt to define a concept with such an ever-changing background is bound to be inadequate. Therefore, the various theories of drug action mentioned above will not be discussed any further. The present work will be discussed only in terms of the concept of occupation of receptors, with the response dependent upon affinity and intrinsic activity.

B. METHODS FOR DIFFERENTIATION OF SPECIFIC DRUG RECEPTORS

A natural consequence of the receptor theory is that drugs should be classified according to the receptors on which they act. Each tissue probably has a limited complement of receptors and an important aim of pharmacological research is to identify the different varieties of these receptors.

The problem of differentiating the receptors for agonists having opposite effects on a tissue does not usually present much difficulty. On the other hand, one is confronted with a relatively difficult problem when two agonists produce similar effects in a preparation, as such effect may be the resultant of the interaction of drugs with the same or quite different receptors. Commonly used methods for differentiating specific receptors in in vitro preparations will be described under four different headings. No mention will be made of in vivo tests as such methods do not necessarily test actions at the receptor level (Ariens, van Rossum and Simonis, 1964).

1. Methods in which antagonists are used.
2. Methods in which agonists are used.
3. Specific desensitization.
4. Receptor Protection

1. Differentiation by Antagonists: Much of the evidence for the specificity of receptors comes from the selectivity of a variety of antagonists. According to Schild (1957), receptors are best identified by antagonists. It must be pointed out that complete specificity of antagonism is rather rare. Atropine, which is a competitive antagonist of acetylcholine over a one thousand-fold range of concentration, is also a competitive antagonist of histamine. This, however, occurs over a narrower range and only in higher concentrations (Arunlakshana & Schild, 1959). Another example is that of β-haloalkylamines, described by Nickerson (1949) as adrenergic blocking agents with a high specificity, also antagonize 5-HT, histamine, and acetylcholine although higher concentrations are required. The antagonism of all these agents by Dibenamine has been shown to be at the receptor level (Furchgott, 1954). Similarly many

examples of unselective antagonism shown by various groups of drugs such as antihistaminics, phenothiazines, etc. may be described. All these examples point out the need for a measure of selective antagonism.

The effect of the antagonist may be measured in terms of the percentage reduction of the recorded response to the agonist. This is possible only in a limited range of doses of the antagonist and is unlikely to give constant results (Gaddum, Hameed, Hathway and Stephens, 1955). Rocha e Silva and his colleagues (1948, 1950) suggested that the effect of an antagonist should be measured in terms of the time the muscle takes to recover when the antagonist is removed from the bath. The work of Fleckenstein (1952) has shown that the action of specific antagonists often lasted longer than those of less specific drugs. However, duration of action alone may not provide sufficient evidence. Mepyramine antagonizes the action of histamine on the guinea-pig ileum in lower doses than promethazine, but its action does not last so long (Reuse, 1948). If the parameter proposed by Rocha e Silva *et al* were used as the only criterion of the action of antagonists, such differences would be obscured. For these reasons, more reliable measures of the effect of an antagonist are used. These are pA_x and Dose-Ratio introduced by Schild and Gaddum respectively.

Schild (1947) defined pA_x as the negative log to the base 10 of the molar concentration of an antagonist which will reduce the effect of a multiple dose (x fold) of an active drug to that of a unit dose. The pA_x 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 (Schild, 1957). To estimate the pA_x it is necessary to expose pieces of tissue to various concentrations of the anta-

gonist and to estimate in each case whether the response to the multiple dose x is greater or less than the required value. Schild (1947) estimated the pA_2 by first getting a constant effect with a constant dose of agonist alone, and then testing the effect of double this dose in the presence of a suitable concentration of the antagonist, and observing whether the effect produced was greater or less than the original effect. The concentration for equal effects was then estimated by interpolation. pA_x values vary with the time for which the tissues have been exposed to the antagonist. Hence, it is essential to mention the exposure time when pA_x values are being reported.

If pA_x values are accurately determined by the method of Schild or by some other satisfactory method (Rocha e Silva, 1959) from a wide range of concentrations of the competitive antagonist, they can help in the classification of drugs according to the receptors on which they act in a given tissue. If different agonists give the same pA_x values for the same antagonist on a given tissue, it is strong provisional evidence that they act on a common receptor. By this criterion histamine, pyridylethylamine, and pyrazoleethylamine, agonists which differ markedly from each other in intrinsic activity, were deduced to be acting on the same receptors in guinea-pig ileum, since they gave the same pA_x values with the same antihistaminic agent (Arunlakshana and Schild, 1959). Other examples of classification of drugs by this procedure are available in literature (van Rossum and Ariens, 1959a; Jenkinson, 1960). However, there are situations where erroneous conclusions may be drawn. pA_x of an antagonist against two agents may be the same and yet one may be acting directly and the other indirectly, e.g., acetylcholine and tetramethylammonium. Another situation in which this principle may break down is

where two agonists act on the same receptor for the effect being studied but one of them can act, in addition, on another set of receptors, so that the effect being measured in this case is the algebraic sum of the effects through the two types of receptors.

Arunlakshana and Schild (1959) also found that the pA_x values of atropine against acetylcholine were similar on such varied organs as frog heart, chick amnion, guinea-pig lung, and mammalian intestine, while they were different in frog rectus muscle. According to these authors, antagonists could thus be used for the comparison of receptors of different tissues and species.

Gaddum et al (1955) measured the effects of active and specific antagonists in terms of dose-ratio, i.e., the ratio of the dose of the agonist causing an effect in the presence of an antagonist to the dose causing the same effect in the absence of the antagonist. When the log-dose-response curves in the presence and absence of the antagonist are parallel, dose-ratio is constant, and equal to the antilogarithm of the distance between the curves. When these curves are not parallel, the dose-ratio varies. The arbitrary convention may then be adopted that the comparison should be at the points corresponding to 50% of the maximum effect, and if this maximum is lessened by the antagonist the measurement should be made at the point corresponding to 50% of this smaller maximum (Schild, 1949). The dose-ratio generally increases with the time of exposure to the antagonist and eventually reaches an approximately constant value, but it may continue to increase over long periods. A standard time of exposure is often adopted to avoid the errors inherent in very long experiments.

The relationship between pA_x and dose-ratio can be appreciated

if it is realized that they both measure the same property, though in different terms. As described already, in the case of pA_x the concentration of the antagonist is worked out, requiring a fixed increase (x fold) in the dose of agonist to produce equal effect. In the case of dose-ratio the increase required in the dose of the agonist to produce equal effect is worked out in the presence of fixed concentration of the antagonist. In either method, the effect measured before and after the antagonist has to be the same, which implies the occupancy of the same number of receptors.

Selective antagonism as measured by pA_x or dose-ratio methods is a reliable test for receptor specificity in most instances. Sometimes, antagonism of agonists may not be selective or may present a border-line situation, thus requiring resolution of the problem by other methods. In such situations, the methods to be described below may attain special significance.

2. Differentiation by Agonists: Agonists may be used in the following ways to show receptor specificity.

a. Partial Agonists: If two agonists act on the same type of receptor, the one with lower intrinsic activity can antagonize the effects of the agonist with higher intrinsic activity, by competing for the receptors. Such compounds with lower intrinsic activity thus show "dualism in action" (Ariens, van Rossum and Simonis, 1957). Stephenson (1956) called such compounds "partial agonists". Ariens and colleagues have shown with several homologous series of compounds that gradually increasing modification of structure of an agonist leads first to compounds possessing dualism in action, and then to competitive antagonists without intrinsic activity. The series which have been studied include drugs

which act on cholinergic receptors in parasympathetic effectors (van Rossum and Ariens, 1959b; van Rossum, 1962a); in ganglia (van Rossum and Ariens, 1959c; van Rossum, 1962b); and in skeletal muscle (van Rossum and Ariens, 1959a); on histamine receptors (Ariens and Simonis, 1960); and on adrenergic receptors of both α- and β-types (Ariens, 1960, 1963).

Partial agonists can induce only a fraction of the maximal effect to be induced by a pure agonist even if they occupy all the available receptors. They have intermediate intrinsic activity which implies that they may act as a competitive synergist and as a competitive antagonist when studied in combination with a pure agonist. This is due to mutual interference in receptor occupation by agonist and partial agonist. If various dose-response curves are made of the partial agonist in the presence of constant but increasing concentrations of pure agonist, a characteristic family of dose-response curves is obtained, the study of which shows that with low concentrations of the pure agonist, the partial agonist acts as a synergist but that with a higher concentration a competitive antagonism is observed. By such studies on certain parasympathetic effector organs, van Rossum (1960, 1962a) found that pilocarpine behaved as a partial agonist, as compared with acetylcholine and furtremethonium, and suggested that some of the conflicting reports in the literature on the effects of pilocarpine may be due to its partial agonistic properties.

The demonstration of partial antagonism among structural analogues can be taken as provisional evidence of action on common receptors for the members of the series. A study of the combined action of pure agonists with partial agonists, as outlined above, may well serve to determine whether they occupy the same receptor sites. However, in

practice this approach has not been widely utilized to differentiate the type of receptors for new compounds, perhaps because the procedure is tedious, especially in tissues which respond slowly to drugs.

b. Drug Synergism: The terms synergism, potentiation, etc., used in connection with the study of drug combination did not have the same meaning with different authors. During recent years, the analysis of the mode of drug antagonism has been an object of great interest and drug synergism has only been remarked in passing (Ariens, van Rossum and Simonis, 1957). Veldstra (1956) mentioned the relation between drug receptor and synergism in his extensive review on "Synergism and Potentiation". Recently (1964) Takagi and Takayanagi have shown that drug synergism may be of great value in differentiating drug receptors, when it is considered in addition to the drug antagonism. They have developed theoretical equations based on mass-action equilibria for the interactions of drugs which produce similar pharmacological actions by acting on the same receptors as well as for drugs which produce similar responses by acting through separate receptors. They have shown a number of experimental examples which fitted well with theory. This seems to be a promising approach which as yet has not been fully explored.

c. Superposition of Maximal Contraction: The theoretical background of this method is that agonists acting through different receptors on a tissue should not interfere with each other's effects. If receptors for an agonist are saturated by concentrations which produce the maximal effect, addition of the second agonist acting through different receptors may cause further contraction. If the second agonist is acting through the same receptors as the saturating agonist no further contraction will be produced. Kuenzle (1960) who advocated this approach for

confirming the existence of specific receptors in a tissue has been able to show specific receptors for acetylcholine, histamine, and adrenaline in the rabbit aorta. However, this method has little practical applicability on account of various limitations such as limited mechanical contractility, inability to maintain a sustained contraction throughout a long period of testing, and unspecific desensitization produced by large concentrations of an agonist.

3. Specific Desensitization: When a tissue is exposed to a high concentration of an agonist, it may rapidly become insensitive to drugs. If the insensitivity is limited to the desensitizing agent itself or to the class of drugs to which this drug belongs, then the desensitization is considered to be specific. On the other hand, if the tissue becomes insensitive to several groups of drugs, the desensitization is considered unspecific. This unspecific desensitization may occur with high concentrations of a drug which causes specific desensitization when used in moderate concentrations.

Specific desensitization is considered to be a receptor phenomenon and is not due to interference with the contractile mechanism, as the tissues respond normally to agonists acting through receptors other than those on which the desensitizing agent acts. Unspecific desensitization does not appear to be a receptor phenomenon, but may be related to the contractile mechanism, perhaps due to loss of intracellular potassium (Paton, 1961).

Barsoum and Gaddum (1935) used specific desensitization as a specific test for histamine in tissue extracts. Gaddum (1953) used this phenomenon to show that 5-hydroxytryptamine combined with tryptamine receptors which were discrete from receptors for acetylcholine, histamine

and substance P. Since then this technique has been used for differentiating receptors for new drugs particularly in cases where the most commonly used method of selective antagonism is inapplicable. Guinea-pig isolated ileum is perhaps the best tissue to differentiate drugs by this method. Some isolated tissues such as strips from rabbit aorta and dog urinary bladder may present practical difficulties on account of their failure to become fully desensitized, i.e., to relax completely if continuously exposed to large concentrations of agonist.

4. Receptor Protection. Selective receptor protection was used by Furchgott (1954) to distinguish between the acetylcholine, histamine, 5-hydroxytryptamine, and adrenaline receptors of smooth muscle in strips of rabbit aorta. He used large concentrations of individual agonists for selective protection against blockade by Dibenamine, a non-equilibrium antagonist. In general, receptor protection is considered to be specific, which means that cross-protection will occur only between drugs which act on the same receptors. Hence, not only high concentrations of agonist but also high concentration of chemically related agonists or reversible competitive antagonists will protect against nonequilibrium blockade (Furchgott, 1955; Ariens, van Rossum and Koopman, 1960).

The use of cross protection experiments against irreversible phenoxybenzamine blockade has been applied by Innes in an attempt to differentiate receptors in certain smooth muscle preparations (Innes, 1962a, 1962b, 1963). In these studies he has shown that (a) ergot alkaloids, ergotamine and ergonovine, act on adrenaline α receptors in the rabbit uterus, rabbit aorta and dog retractor penis, (b) the direct component of the stimulating effect of 5-hydroxytryptamine on cat spleen strips is produced through α receptors and (c) dexamphetamine acts on 5-HT re-

ceptors to produce its stimulatory effect on rat stomach, dog retractor penis, rabbit uterus, rabbit aorta, and guinea-pig ileum. In these experiments, Innes based his conclusions regarding the type of the receptors concerned in the action of the test agonists not only on the demonstration of cross-protection between the test agonist and the type-specific agonist, but also on the lack of cross-protection between the test agonist and the agonists specific for other receptors.

The protection technique depends on the assumption that the protecting agent in high concentrations occupies a large proportion of the type-specific receptors, thus preventing access of the blocking agent to these receptor sites but not to the receptor sites for other types of agonists. Protection technique is considered to be the most direct approach for the demonstration of specificity of receptors (Ariens and Simonis, 1962). However, Waud (1962) has recently criticized the validity of cross-protection technique in identifying drug receptors, considering it to be unsound. He has pointed out that (a) the agonist used as a protecting agent may have some affinity for receptors other than the specific type through which it initiates a response; and (b) an agonist in sufficient concentration may still elicit a marked response even when a major fraction of its receptors are irreversibly blocked (spare receptors). He argued that partial protection of response to a second agonist by a first agonist is not convincing evidence that both elicit responses through a single type of receptor. His criticism seems to be well-founded in the light of evidence presented by Kohli (1965) that 5-hydroxytryptamine provides some protection for adrenaline receptors in rabbit aorta. However, it appears that the cross-protection method is quite useful provided conclusions regarding the type of recep-

tors concerned in the action of the test agonists are based not only on the demonstration of cross-protection between the test agonist and type-specific agonist but also on the lack of cross-protection between the test agonist and the agonists specific for other receptors.

CHAPTER 3

5-HYDROXYTRYPTAMINE (5-HT) RECEPTORS

A. 5-HT RECEPTORS IN THE GUINEA-PIG ILEUM

'M' and 'D' Receptors

B. 5-HT RECEPTORS IN OTHER TISSUES

Dog Urinary Bladder

Smooth muscle is known to have specific receptors for at least four types of agonist, acetylcholine, histamine, 5-hydroxytryptamine and catecholamines (Furchgott, 1954). The pharmacological characteristics of receptor sites sensitive to acetylcholine, histamine and catecholamines are well known. It is beyond the scope of the present work to review at length the literature concerning these agonists. However, the investigations regarding the role and characteristics of the receptors sensitive to 5-HT are directly related to the problem investigated in the present study. Pertinent literature is therefore reviewed.

Our knowledge of 5-HT receptors is based largely on the study of few simple smooth muscle preparations, in which mainly the effects of a variety of 5-HT antagonists have been investigated. The results obtained with such preparations, however, have proved to be difficult to interpret.

A. 5-HT RECEPTORS IN THE GUINEA-PIG ILEUM

In 1953 Gaddum demonstrated that exposure to high concentrations of 5-HT or tryptamine abolished responses of the guinea-pig to both of them without markedly altering responses to histamine or substance P. He therefore suggested that tryptamine and 5-HT act on the same receptors, which were distinct from receptors for other agonists.

Rocha e Silva, Valle and Picarelli (1953) showed that the effect of 5-HT in guinea-pig ileum was blocked by atropine or cocaine, and was not modified by hexamethonium. Robertson (1953) also made similar observations. Rocha e Silva *et al* (1953) suggested that 5-HT acted on the postganglionic cholinergic fibres of the intramural nervous system.

Gaddum and Hameed (1954), seeking further support for the hypo-

thesis of specific receptors for 5-HT, studied the interactions between a number of agonists and antagonists on rat uterus, guinea-pig ileum, and rabbit ear vessels. They showed that smaller concentrations of mepyramine, piperoxan, or atropine which inhibited the effects of histamine, adrenaline, and acetylcholine respectively did so without altering responses to 5-HT. From these experiments they concluded that 5-HT produces its effects by acting on specific receptors. High concentrations of atropine, however, were found to antagonize 5-HT. They explained this antagonism as inhibition of the action of endogenous acetylcholine released from the nerve endings.

Gaddum and Hameed (1954) studied a large number of 5-HT antagonists, including various indole and tryptamine derivatives. They found that 5-HT antagonists gave different results when tested on different preparations. In rat uterus and rabbit ear vessels, various lysergic acid derivatives were found to be very potent antagonists of 5-HT while atropine and cocaine were not so active. Self desensitization by 5-HT was difficult to demonstrate in either of these preparations. On the other hand, in guinea-pig ileum atropine, cocaine and large concentrations of 5-HT were found to be more active as antagonists of 5-HT than the derivatives of ergot. In order to explain such facts, they suggested that there were two kinds of 5-HT receptor: one in the smooth muscle of the rat uterus and the rabbit ear vessels, which are easily blocked by LSD, gramine or dihydroergotamine; and another in the intestine, not easily blocked by such drugs.

Theory of M and D Receptors. In the various studies (Gaddum, 1953a; Feldberg and Toh, 1953; Robertson, 1953; Rocha e Silva et al., 1953) in which antagonism by atropine of 5-HT action on guinea-pig ileum

has been investigated, marked divergence in results was reported. In view of conflicting reports, Cambridge and Holgate (1955) undertook a careful study of atropine 5-HT interactions. Acetylcholine and histamine were used as control drugs in this study. When the responses of guinea-pig ileum to a constant dose of agonists were plotted against the log dose of atropine, acetylcholine and histamine gave simple S-shaped curves, but with 5-HT there was a plateau over the range of concentrations 0.1 to 1.0 μg/ml, i.e., increasing the concentrations of the antagonist by one hundred fold in this range did not increase the depression of responses to 5-HT. Earlier, Gaddum and Hameed (1954) had made a similar observation. They had found that increasing by 100 times the concentration of LSD which diminished the response of guinea-pig ileum to 5-HT by 50% did not increase the effect.

Gaddum and Picarelli (1957) suggested that these observations could be explained on the theory that the two types of receptor postulated by Gaddum and Hameed were both present in the guinea-pig ileum and drugs may block one type of receptor without affecting the other type. Thus atropine in low concentrations blocked the cholinergic part of the response to 5-HT mediated through its action on the nervous elements. The other part of the response to 5-HT was produced through receptors on the smooth muscle itself and was comparatively resistant to atropine. Similarly LSD at low concentrations blocked the latter part of the 5-HT response and higher concentrations had no more effect on the residual (cholinergic) response to 5-HT. This theory appeared to be supported by the observation of Kosterlitz and Robinson (1955) that morphine only partially inhibited the response of guinea-pig ileum to 5-HT.

Gaddum and Picarelli (1957) supported their theory by presen-

ting the following evidence. They tested the effects of various antagonists on guinea-pig ileum in the presence of either morphine or phenoxybenzamine. The antagonistic potency against 5-HT was expressed in terms of dose-ratio. The results showed that the antagonists fell into two groups. For one group the dose ratios of 5-HT were 1-4 in the presence of morphine and 50-400 in the presence of phenoxybenzamine. The dose ratios exhibited by 5-HT for the other group were just the reverse, i.e., 30-500 in the presence of morphine and 1-6 in the presence of phenoxybenzamine. Hence Gaddum and Picarelli suggested that the drugs such as atropine, cocaine or methadone, which showed only weak antagonistic activity in the presence of morphine and retained high antagonistic activity in the presence of phenoxybenzamine caused inhibition through 'M' receptors, probably in the neuronal tissue; while drugs like phenoxybenzamine, dihydroergotamine, LSD, and BOL, which exhibited high antagonistic activity in the presence of morphine and only little in the presence of phenoxybenzamine, acted on 'D' receptors, probably in the muscle.

Kosterlitz and Robinson (1958), investigating the inhibitory action of morphine on the contraction of longitudinal muscle coat of the guinea-pig isolated ileum, found that their results with morphine were consistent with the hypothesis that 5-HT acted on two different sites on guinea-pig ileum.

Many studies regarding further differentiation of the 5-HT receptors in the guinea-pig ileum and their location in the nervous tissue have been reported. It has already been mentioned that nervous receptors of 5-HT in the guinea-pig ileum seem to differ from the ganglionic cholinergic receptors of this organ in that they are not suppressed by hexamethonium or by a large blocking dose of nicotine (Rocha e Silva, 1953).

Antagonism studies with morphine suggested that the sites are probably peripheral to the autonomic ganglia but central to parasympathetic receptor sites, since Paton (1957) showed that morphine inhibits the release of acetylcholine. However, recent studies which showed that 5-HT in small quantities produced facilitation and also direct stimulation of the sympathetic ganglia the cat (Trendelenburg, 1956; Hertzler, 1961), have suggested, by analogy, that in the guinea-pig ileum nervous receptors may also be located at the intramural autonomic ganglia, although these receptors function differently from the cholinergic ganglionic receptors.

Day and Vane (1963) tested the hypothesis of 'M' and 'D' receptors in guinea-pig ileum by comparing the block of 5-HT by morphine or anoxia with the block of coaxial stimulation. When anoxia completely blocked coaxial stimulation it was assumed that the neuronal elements were no longer excitable. If a drug still evoked responses in the strips under such anoxic conditions, it was then believed to be acting on the muscle cells directly. Their results showed that in anoxic strips the dose of 5-HT had to be increased some 500-fold to produce a response equal to a response under normal conditions while anoxia hardly modified the effects of acetylcholine or histamine. In the presence of morphine, on the other hand, the dose of 5-HT had to be increased only 2- to 13-fold and coaxial stimulation was still effective. Moreover, if anoxia was caused after morphine treatment, a further increase in the concentration of 5-HT by about 400-fold was required to evoke responses equal to the controls. They therefore concluded that (i) 5-HT contracts the longitudinal muscle of guinea-pig ileum mainly through the receptors in the nervous tissue, (ii) morphine can block only part of this response, and (iii) the smooth muscle receptors are of negligible importance under

normal conditions. In view of the effectiveness of phenoxybenzamine as an antagonist of 5-HT in the guinea-pig ileum, these authors suggested that in this organ the majority of the 'D' receptors are located in the nervous tissue. They also proposed that the terms 'M' and 'D' receptors should be used only to indicate their sensitivity to the specific antagonists and should not be quantitatively equated with nervous and smooth muscle receptors.

Harry (1963) who used circular muscular strips of guinea-pig ileum to study the site of action of 5-HT, found that the effect of 5-HT as well as of nicotine and histamine was antagonized by a variety of blocking agents such as atropine, morphine, botulinum toxin (Type A), hemicholinium, and procaine. He therefore, concluded that all three drugs, 5-HT, nicotine, and histamine, acted on the intramural neuronal plexus in the circular muscle strips, a rather unusual site of action for histamine, which is known to have a direct action on the longitudinal muscle. He further showed that excess 5-HT blocked effects of 5-HT only, mepyramine or excess histamine blocked the effect of histamine only, and hexamethonium blocked effects of nicotine only, thus supporting the hypothesis of specific 5-HT receptors.

Brownlee and Johnson (1963) working with longitudinal muscle segments of guinea-pig ileum, came to a similar conclusion. They once again confirmed the high specificity of self desensitization by 5-HT. By using hyoscine and a number of ganglion blocking agents with depolarizing, competitive, or mixed type of action, they presented strong evidence for the localization of the site of action of 5-HT. Competitive blocking agents did not modify the effect of 5-HT but the depolarizing agents did,

as did hyoscine. They therefore concluded that 5-HT activated specific receptors in the intramural parasympathetic ganglion cells.

Recently, Paton and Aboo Zar (1965) have used a denervated preparation of the longitudinal muscle of the guinea-pig ileum, to analyse the site of action of various agonists. They have shown that acetylcholine and histamine act principally directly on the smooth muscle whereas 5-HT acts largely through the nerves. This work, which is still in progress, is consistent with earlier studies and is likely to further elucidate the exact site of action of 5-HT in guinea-pig ileum.

B. 5-HT RECEPTORS IN OTHER TISSUES

Effects of various antagonists on the response of 5-HT in different preparations revealed close similarity between rat uterus and vascular smooth muscle (Gaddum and Hameed, 1954). The relative resistance of rat uterus to block of the 5-HT response by antagonists such as atropine or cocaine compared with high sensitivity to antagonists like Dibenamine and LSD led to the conclusions that 5-HT receptors in the uterine smooth muscle were 'D' type. The direct test of specificity of 5-HT, i.e., specific desensitization by excess of 5-HT was found to be difficult but possible to demonstrate in this organ.

In a similar manner, antagonism studies by Vane (1957) revealed predominence of 'D' type receptors in the rat stomach. However, Vane (1960) presented evidence that the 5-HT receptors in rat stomach were less specific, since sympathomimetic amines of the amphetamine type reacted with the same receptor as 5-HT. Innes (1962) who used selective receptor protection technique confirmed that in a number of tissues amphetamine

and 5-HT acted on the same receptors. Paton and Vane (1963) showed that the majority of 'D' receptors were present in the smooth muscle of rat stomach.

In vascular smooth muscle, where both 5-HT and catecholamines have a vasoconstrictor effect, clear cut differentiation by means of antagonists has not been always easy. Some antagonists (LSD, BOL) exhibit a degree of selectivity that indicate receptor specificity. Yet structurally related drugs such as ergotamine and dihydroergotamine show equal antagonism of 5-HT and catecholamines, whereas other congeners (ergonovine) show no antagonism to either agonist. Selective receptor protection (Furchgott, 1954) has proved a valuable new tool to differentiate the receptors. Results of Furchgott's studies with rabbit aorta constitute reasonable evidence for specific 5-HT receptors in that organ.

Innes (1962) made an interesting study of the action of 5-HT on cat spleen. He showed that 5-HT had two actions, neither of which was on receptors that were specific for 5-HT. The main action of 5-HT was shown to be due to release of stored noradrenaline as sensitivity to 5-HT of spleen strips from cats treated 24 hours earlier with reserpine was found to be only one fiftieth of that of normal strips. The direct action of 5-HT was on adrenaline receptors. This conclusion was based primarily on demonstration of marked cross-protection between these two agonists against block by phenoxybenzamine. In addition, strips desensitized by large doses of 5-HT responded normally to histamine or acetylcholine but were unresponsive to adrenaline or 5-HT.

Koella and Schaeppi (1962) and van Alphen, Robinette and Macri (1964) compared the response of intact iris and isolated dilator and

sphincter pupillae to catecholamines and 5-HT. The effect of 5-HT was similar to that of acetylcholine and opposite to those of catecholamines. These effects of 5-HT were resistant to doses of atropine which abolished acetylcholine effects. These observations point to the existence of specific 5-HT receptors in the iris muscles.

Specific 5-HT receptors in the nictitating membrane was postulated by Thompson (1958) who showed that LSD antagonized the effects of 5-HT without inhibiting the effects of acetylcholine or noradrenaline. However, responses to 5-HT were also antagonized by atropine, haloalkylamines and dihydroergotamine. Further, desensitization with tryptamine led to almost equal depression of the responses to 5-HT and catecholamines. Schaeppi (1963) found that phentolamine inhibited responses to noradrenaline only slightly more than those to 5-HT. In view of these observations, it is not possible to arrive at definite conclusion regarding specific 5-HT receptors in the nictitating membrane.

The effect of 5-HT in bronchial muscle is selectively antagonized by BOL and is relatively resistant to atropine and antihistaminics. Catecholamines, however, act as physiological antagonists. These observations support the suggestion of specific 5-HT receptors in this tissue (Konzett, 1956). Recently, Constantine and Knott (1964) have suggested that the effect of 5-HT on guinea-pig trachea does not depend upon the activation of a cholinergic mechanism as it does, in part, in guinea-pig ileum. They have presented evidence which shows that 5-HT receptors in guinea-pig trachea resemble 'D' (muscle) receptors in that they are not blocked by atropine or by morphine, but are blocked by methysergide.

Dog Urinary Bladder. Only a few studies of 5-HT on the smooth

muscle of urinary bladder have been made. Erspamer (1953) showed that 5-HT stimulates the dog isolated urinary bladder and is, in this respect, a more powerful stimulant than any other biogenic amine. He reported that Dibenamine markedly antagonized 5-HT elicited contractions of isolated bladder. Prior administration of Dibenamine in anesthetized dogs reduced but did not completely abolish the response of the urinary bladder in situ to the intravenous injection of 5-HT.

Urinary bladder has been found to differ from many other visceral organs in respect to its response to a variety of cholinergic stimuli (Henderson and Roepke, 1935; Ursillo and Clark, 1956; Gyermek, 1961). Gyermek (1961) who studied the pelvic nerve-bladder preparation in situ of the dog, postulated that there were two types of cholinergic receptor sites in the bladder, one of them being a typical postganglionic parasympathetic receptor, and the other an atypical peripheral ganglion with an endplate-like function.

Using this preparation, Gyermek showed that intra-aortic injection of 5-HT produced contraction and enhanced the action of various stimuli (pelvic nerve stimulation, DMPP, methacholine, acetylcholine, KCI. The bladder contraction produced by 5-HT was highly resistant to atropine or hexamethonium. It was concluded that 5-HT does not act directly or indirectly (through the liberation of acetylcholine) on the cholinergic receptor sites of the bladder.

Gyermek (1962) observed that the stimulant action of 5-HT was composed of two phases. The first phase consisted of a fast, twitch like contraction followed by a phase of slow prolonged contraction. The first phase was inhibited by morphine, cocaine and large doses of 5-HT but was unaffected by BOL. The slow phase, on the other hand, showed just the

opposite sensitivities to the two types of antagonists. Gyermek, therefore, postulated the presence of both types of receptors in this organ, 'M' receptors responsible for the fast phase and 'D' receptors responsible for the slow phase.

CHAPTER 4

STATEMENT OF THE PROBLEM

STATEMENT OF THE PROBLEM

The subject of investigation in this thesis is to identify the receptors excited by ergot alkaloids to cause stimulation in tissues normally inhibited by noradrenaline.

The close structural relationship between ergot compounds and 5-HT due to the presence of an indole nucleus, and specific antagonism between ergot derivatives and 5-HT in many smooth muscle preparations are highly suggestive that the stimulatory effect of ergot compounds may be due to their interaction with 5-HT receptors.

It was decided to test this hypothesis in in vitro preparations, where receptor identification techniques can be applied. The tissues chosen for this study were guinea-pig ileum and dog urinary bladder. These tissues satisfied the following criteria:

a) noradrenaline normally inhibits.

b) sensitive to 5-HT.

A drug may activate receptors of a given type by reacting directly with such receptors or by acting indirectly to cause within the biological test system the liberation, formation or accumulation of a second substance, which then activates these receptors (Furchgott, 1964). Some drugs appear to activate receptors in a single type of effector cells by both direct and indirect actions (Innes, 1962).

Taking into consideration the above mentioned statements, the following mechanisms might be involved in the pharmacological actions of the ergot compounds under study: (i) these compounds may cause the release of an endogenous stimulatory substance; (ii) the receptors for

other known excitatory agonists, e.g., acetylcholine, histamine or 5-hydroxytryptamine may be the site of action of these compounds; (iii) yet another set of receptors may be involved.

According to the current concept, both α and β receptors for catecholamines subserve inhibition in intestinal smooth muscle (Ahlquist and Levy, 1959; Kosterlitz and Watt, 1964). Therefore the ergot alkaloids causing contraction of guinea-pig ileum cannot do so by activating adrenergic receptors. Vane (1960), Innes (1963) and Kohli (1965) have shown the involvement of 5-HT receptors in the action of some stimulatory sympathomimetics in different preparations of the gut. If 5-HT receptors are actually involved in the action of ergot compounds, then the scope of this investigation becomes more specific, i.e., whether ergot compounds activate 'M' or 'D' or both types of 5-HT receptors.

As indicated in Chapter 4, the differentiation and specificity of 5-HT receptors in dog urinary bladder is not well documented. Preliminary work regarding the specificity of 5-HT receptors in this tissue had to be done, before the study of mechanism of action of ergot compounds could be undertaken. After having established the specificity and characteristics of 5-HT receptors in the dog urinary bladder, a pharmacological analysis of the mode of action of ergot compounds was carried out.

SECTION II

METHODS AND MATERIALS

CHAPTER 5

METHODS AND MATERIALS

A. GUINEA-PIG ILEUM

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Desensitization Studies

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Antagonistic Activity of Ergotamine and Dihydroergotamine

B. DOG URINARY BLADDER

General Procedures

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C. DRUGS AND SOLUTIONS

The ergot alkaloids fall into two distinct groups on the basis of their chemical composition as well as their better known pharmacological actions (see page 4). Out of the large number of available ergot compounds, four drugs representing natural as well as synthetic derivatives of the two groups, were selected for the present study. These are ergonovine and its synthetic derivative, methylergonovine belonging to one group, and ergotamine and its derivative, dihydroergotamine, belonging to the other group.

After a preliminary study of the ergot compounds on various isolated smooth muscle preparations where the action of adrenaline is inhibitory, guinea-pig ileum and dog urinary bladder were selected for detailed study to determine the type of receptors on which the ergot alkaloids act. Non-terminal segments of the ileum were used as the terminal segments are known to respond to adrenaline by contraction (Munro, 1952).

The experimental procedures used in this study are described below.

A. GUINEA-PIG ILEUM

General Procedures. Guinea-pigs of either sex weighing 250-500 g were fasted overnight and killed by a blow on the head. Unless otherwise specified, non-terminal ileum was used and this will be referred to as guinea-pig ileum throughout. About fifteen cm of ileum immediately proximal to the ileo-caecal junction was rejected and a length of the adjacent ileum removed to prepare the isolated segments. Mesenteric attachments were cleared and sections of about 2.5-3 cm in

length were each suspended in a 10 ml bath containing Krebs-Henseleit solution (see page 60).

A gas mixture of 95% O_2 and 5% CO_2 was bubbled through the main reservoir of bathing fluid as well as through the bath fluid which was kept at 38 ± 0.2°C. Isotonic contractions of the gut against 1 g tension and amplified 5.5 fold were recorded on a kymograph. Tissues were suspended for about an hour before testing any drugs. The bathing fluid was changed every fifteen minutes during this period.

Before starting the actual experiment, the effect of 10^{-6} g/ml noradrenaline was tested in each preparation to ascertain the inhibitory response of the tissue to it. Unless otherwise specified, responses of the tissues to the various agonists were tested at intervals of ten minutes. Each dose of the agonist remained in the bath until the full contraction for that dose was attained, usually less than two minutes after the drug was added. The bath was then emptied and the fluid replaced. The fluid was exchanged at five minute intervals between drug additions.

Experiments. In preliminary experiments, full dose-response curves were made for each of the agonists, acetylcholine, histamine, 5-HT, ergonovine, and methylergonovine. These curves showed the comparative activity of the various agonists and served as guide for selecting various doses of the agonists in future experiments.

Antagonism Studies. In most experiments, a dose-response curve was made for 5-HT, acetylcholine and/or histamine and either ergonovine or methylergonovine. In some experiments, all the agonists were included. Usually three or four doses of the agonist whose effects were on the ascending parts of full dose-response curves as indicated by the preliminary experiments, were employed. In order to avoid errors due to

persistence of effects, no test for a new agonist was done within 30 minutes of the last dose of the preceding agonist. The dose-response curves were then repeated in the presence of the antagonists. The concentration of the various antagonists was maintained in the bath by adding fresh doses of the antagonists whenever the fluid was changed. However, there were two exceptions from the usual procedure. In case of the non-equilibrium antagonist phenoxybenzamine, the tissues were given only a single exposure of 5 minutes to the drug which was then washed out. The responses of the tissue to the various doses of the agonists were then tested. With BOL the concentration was not maintained throughout the period of experiment as the antagonistic effect of BOL goes on changing rapidly on continuous exposure. The tissue was exposed to the selected concentration of BOL for five minutes before each dose of the agonist was tested.

The dose-response curves for the agonists in the absence and presence of various antagonists, atropine sulphate, cocaine hydrochloride, morphine sulphate, BOL, phenoxybenzamine hydrochloride, phentolamine hydrochloride, hexamethonium bromide, dimethylphenylpiperazinium iodide (DMPP), and diphenhydramine hydrochloride, were compared in order to differentiate the site of action of various agonists. In appropriate cases, the effects of the antagonists were measured in terms of dose-ratio (Gaddum et al., 1955). The dose-ratio is the ratio of equiactive doses in the presence and absence of the antagonist (Chapter 3).

Desensitization Studies. Guinea-pig ileum can be specifically desensitized to 5-HT and tryptamine by exposure to high concentrations of either drug (Gaddum, 1953; Rocha e Silva et al., 1953). This phenom-

enon of cross-desensitization has been used in the present study to identify receptors for ergot compounds.

Preliminary experiments were done to find out the dose of 5-HT which would render the tissue insensitive to 5-HT without affecting the sensitivity of the control agonists, acetylcholine or histamine. In a similar manner, desensitizing doses of ergonovine and methylergonovine, which would render the tissue insensitive to the respective ergot alkaloid itself without affecting the sensitivity to acetylcholine or histamine, was found. These were 10^{-4} g/ml for both ergot alkaloids.

The following procedure was adopted for the conduct of the experiments. In each experiment four adjacent segments of the ileum from the same guinea-pig were used. Out of these, one tissue was used as control while the other three tissues were used to study the effect of the desensitizing agents. A three point dose-response curve was made in all four tissues for each of the agonists (acetylcholine, histamine, 5-HT, ergonovine, and methylergonovine). Then the predetermined desensitizing dose of 5-HT was added to one bath, that of ergonovine to the second bath and that of methylergonovine to the third bath. No desensitizing agent was added to the fourth bath. During the exposure to the desensitizing drugs the bathing fluid was changed every fifteen minutes and the drug concentrations were maintained by immediate addition of the drug after the wash. Usually after exposure for 45 minutes, the tissues were desensitized and no response could be elicited by further addition of the desensitizing agent to the bath. From then on tests were made every 15 minutes for responses to various agonists in the continued presence of the desensitizing agents. Dose-response curves in all four tis-

sues for each of the agonists were repeated and compared to dose-response curves made before exposure of the tissues to the desensitizing agents.

Effect of Cold Storage. Drugs can contract smooth muscle of isolated preparations either by a direct action on the muscle fibres or indirectly through excitation of the intrinsic network of nerves. Most methods used to distinguish between two kinds of action involve the inactivation of nervous tissue in such a way that the responses of the muscle itself are as little altered as possible. In order to inactivate the nervous tissue of guinea-pig ileum, many workers have used ileum which had been stored at 4°C for 24-72 hours (Ambache, 1946; Blair and Clark, 1956; Innes, Kosterlitz and Robinson, 1957). The same procedure has been adopted in the present study.

Responses of the ileum to various agonists were tested at 38°C, a dose-response curve was made for each of the agonists, usually at three dose levels. Then the tissue along with the attached threads was removed from the original bath and placed in a beaker containing cold Krebs-Henseleit solution. The beaker was kept in the refrigerator at 4°C for 24-72 hours. After the period of cold storage, the tissue was again suspended in the organ bath under the same conditions as for the control responses and allowed to equilibrate at 38°C for ninety minutes. The bathing fluid was changed every 15 minutes during this period. Responses of the tissue to the agonists were tested again in the same order. Dose-response curves for each of the agonists were made and compared to curves made before cold storage of the tissue.

Effect of Reserpine Pretreatment. Some drugs may activate receptors of a given type, not by directly reacting with such receptors,

but by causing release of an endogenous stimulatory substance which then activates these receptors, e.g., tyramine causes release of stored noradrenaline which then activates the smooth muscle (Burn and Rand, 1958). This is based on evidence that tyramine has little action on smooth muscle depleted of noradrenaline. The possibility that ergot compounds act indirectly through the liberation of 5-HT was therefore tested. Reserpine, which markedly depletes guinea-pig ileum of its 5-HT contents as well as argentaffin cells (Bulbring and Crema, 1959), was injected intraperitoneally into guinea-pigs in a dose of 1 mg/kg 24 hours before the experiment. A segment from ileum was then set up as already described. A segment from ileum of an untreated animal was also set up as a control. A full dose-response curve for each of the agonists was made in each tissue. The sensitivity of preparations from reserpine pretreated animals was compared to that of normal preparations in respect of their contractile responses to various agonists by plotting the curves which represented the mean of experiments in this series.

Partial Agonists. Some drugs, instead of being either pure agonists or pure antagonists, may possess both agonistic and antagonistic activities. Such drugs have been called 'partial agonists' (Stephenson, 1956) and have been shown to possess intermediate intrinsic activity (Ariens, 1954; Ariens and Simonis, 1954; Stephenson, 1956; Ariens, van Rossum and Simonis, 1957; Ariens, 1964). Their mode of action may become evident if such drugs are studied in combination with a pure agonist. If various dose-response curves are made of the partial agonist in the presence of constant but increasing concentrations of the pure agonist, a characteristic family of dose-response curves is obtained,

the study of which shows that with low concentrations of the pure agonist the partial agonist acts as a synergist but with a higher concentration there is competitive antagonism.

Both ergonovine and methylergonovine have been shown to be potent antagonists of 5-HT in rat uterus (Cerletti and Doepfner, 1958). The intrinsic activity of these compounds in guinea-pig ileum is evident from the contractile response of this tissue to them as shown in the present study. The combined action of 5-HT with ergonovine or methylergonovine in guinea-pig ileum was therefore studied to determine whether these drugs were mutually competitive and whether they occupied the same receptor sites.

In each experiment, a segment of ileum was set up as outlined under general procedure. First of all, responses to graded doses of ergonovine alone were measured and a dose-response curve plotted. Next, responses to the same doses of ergonovine combined with a constant dose of 5-HT (10^{-8} g/ml) were determined and a dose-response curve constructed. In this case, ergonovine and 5-HT were added to the bath simultaneously. Dose-response curves of ergonovine combined with 3 x, 10 x, and 30 x 10^{-8} g/ml of 5-HT were determined similarly. In all, five dose-response curves of ergonovine were made; one curve without any combination of 5-HT and the other four combined with constant but increasing doses of 5-HT.

Dose-response curves for methylergonovine combined with increasing doses of 5-HT were plotted in the same way. Separate preparations were used for ergonovine and methylergonovine.

Antagonistic Activity of Ergotamine and Dihydroergotamine. Er-

got alkaloids possessing a polypeptide side chain can block excitatory responses of various smooth muscle organs to adrenaline (Rothlin, 1947). Rothlin et al (1954) have reported that these alkaloids can also antagonize the adrenaline- and noradrenaline-induced inhibition of the pendular movements of the rabbit isolated intestine. Terminal segments (adjacent to the ileo-caecal junction) of the guinea-pig ileum respond to adrenaline and noradrenaline by contraction, whereas nonterminal segments are inhibited by adrenaline, noradrenaline, and isoprenaline. It was therefore of interest to study the effect of ergotamine and dihydroergotamine on excitatory and inhibitory responses of the different segments of guinea-pig ileum to sympathomimetic amines.

In terminal segments of the ileum, responses to at least three graded doses of adrenaline and noradrenaline were recorded and dose-response curves were constructed. Then ergotamine or dihydroergotamine was added to the bath and allowed to act for 10 minutes before and during the administration of each test dose of adrenaline or noradrenaline. The alkaloid was washed out after the residual response of adrenaline or noradrenaline had reached its maximum. At least 20 minutes were allowed before the next addition of the alkaloid.

In some experiments, the effects of ergotamine and dihydroergotamine on responses to graded doses of acetylcholine, histamine, 5-HT, ergonovine, and methylergonovine were also studied. Dose-response curves for each of the agonists before and after ergotamine or dihydroergotamine were compared.

In case of nonterminal segments, the effect of ergotamine and dihydroergotamine on the inhibitory responses of the ileum to the sym-

pathomimetic amines was investigated by using Paton's (1955) technique of coaxial stimulation. These segments were set up for electrical stimulation through coaxial electrodes. Electrodes were placed within and without the lumen of the segment immersed in Krebs-Henseleit solution. Rectangular current pulses, usually 1 msec duration and of sufficient strength to produce a maximal response (usually about 30 V) to a single shock, were applied at a rate of 6 shocks/min. Vigorous twitch-like contractions of the gut were recorded on the kymograph.

A series of responses to coaxial stimulation was obtained. During coaxial stimulation of the gut, graded doses of noradrenaline and isoprenaline were added to the bath at 20 minute intervals. Percentage decrease in the height of contraction was measured for each dose of noradrenaline and isoprenaline and dose-response curves were constructed. The effect of blocking agents was investigated. The inhibitory responses to the same doses of noradrenaline and isoprenaline were measured first in the presence of ergotamine or dihydroergotamine or pronethalol alone and then during the simultaneous presence of pronethalol and ergotamine or pronethalol and dihydroergotamine. Dose-response curves for these tests were determined and compared to control curves.

B. DOG URINARY BLADDER

General Procedures. Puppies of either sex weighing between 2 and 4 kg were killed by a blow on the head. A midline incision into the lower part of anterior abdominal wall exposed the urinary bladder which was removed from the body after being isolated from the surrounding structures. It was laid on a filter paper soaked in cold Krebs-

Henseleit solution. An incision was made in its anterior wall extending from the urethral opening upwards towards its base, resulting in an almost rectangular flap. Strips 2.5-3 cm long and about 3 mm wide were removed from the flap. These strips were cut either horizontally or vertically from any area of the flap. In preliminary experiments about 10% of horizontal strips responded to noradrenaline by contraction but all vertical strips relaxed. All subsequent experiments were done on vertical strips.

A loop of thread was stitched through one end of each strip and a length of thread through the other. The strip was then mounted in a 10 ml bath containing Krebs-Henseleit solution at 38 ± 0.2°C bubbled with 95% O_2 and 5% CO_2. Isotonic contractions against 1 g tension were recorded on a kymograph by levers adjusted to give 5.5 fold amplification. The strip was allowed to stretch for 90-120 minutes before tests were begun and approximately doubled its initial length during this period. The bathing fluid was replaced every 15 minutes during this waiting period.

Unless otherwise specified, responses of the tissues to various agonists were tested at intervals of twenty minutes. Each dose of the agonist remained in the bath until the full contraction for that dose was attained, usually less than four minutes after the drug was added. The drug was then washed out. Washing was repeated every five minutes till the muscle had relaxed to its precontraction level. No agonist was added within 5 minutes of the last wash.

Experiments. Very few pharmacological reports with isolated urinary bladder as test preparation are available in the literature. Pre-

liminary experiments were therefore done to obtain information about the magnitude of spontaneous activity and the sensitivity to various drugs. Full dose-response curves were made for acetylcholine, histamine, and 5-HT. On account of sluggish responses of the preparation to some drugs and failure to return to the former base line within a reasonable time, it was impracticable to do full dose-response curves for the rest of the agonists. The effect of only 3 graded doses of these drugs was therefore studied.

Antagonism Studies. The following series of experiments were done:

(i) The effect of various antagonists, atropine sulphate, BOL bitartrate, diphenhydramine hydrochloride, morphine sulphate, phenoxybenzamine hydrochloride, and phentolamine chloride, on the responses to various agonists was studied by comparison of dose-response curves of the agonists with and without the antagonists.

In each experiment, a dose-response curve was made for acetylcholine, histamine, 5-HT, ergonovine, and methylergonovine. Only 3 doses of the agonists whose effects were on the ascending parts of full dose-response curves as indicated by the preliminary experiments, were employed. An interval of about 40-50 minutes was allowed between the last dose of the preceding agonist and the first dose of the new agonist, in order to avoid any interference due to persistence of effects of the first agonist. The dose-response curves were then repeated in the presence of the antagonists. The concentrations of the antagonists except BOL and phenoxybenzamine were maintained in the bath during the period of testing antagonism, fresh doses of the antagonists having

been added immediately whenever the bathing fluid was changed. With BOL the concentration was not maintained throughout the period of experiment; instead the tissue was exposed to the selected concentration of BOL for five minutes before each dose of the agonist was tested. In case of phenoxybenzamine the tissues were given only a single exposure of 5 minutes and the responses to the various doses of the agonists were then tested.

(ii) pA_2 determinations with BOL: Earlier experiments indicated that BOL showed marked antagonism against the contractile responses to 5-HT, ergonovine and methylergonovine as compared to its effects on acetylcholine or histamine responses. pA_2 values with BOL were determined to get more exact information regarding this antagonism.

Initial experiments indicated that the concentrations of BOL during the presence of which responses to double the dose of each of the agonists (5-HT, ergonovine and methylergonovine) were equal to their single doses in the absence of BOL, were almost identical and about 10^{-10} g/ml. Concentrations of BOL for similar effects on acetylcholine and histamine were between 10^{-7} and 10^{-6} g/ml. The exact pA_2 was determined by adopting the following procedure.

In most experiments, separate tissues obtained from the same animal were used to determine pA_2 values for different agonists. A dose of the agonist sufficient to give a reproducible submaximal effect was found. Development of desensitization of tachphylaxis was shown not to occur by testing double the dose twice or three times before adding BOL. A dose of BOL predetermined by the initial experiments indicated above was added to the bath and allowed to act for 5 minutes. Double the initial dose of the agonist was then added to the bath and its

effect measured. The drugs were washed out and single doses of the agonist were added until the responses were back to the original size. This was necessary because the effect of BOL continues to increase with prolonged exposure of the tissue to it. If the effect of double dose of the agonist in the presence of BOL was greater or less than the effect of the single dose in the absence of antagonist, the initial procedure was repeated with increasing or decreasing concentrations of BOL respectively. In most experiments, it was possible to determine the accurate pA_2 values for the agonists by this method. pA_2 was then calculated with the molecular weight of BOL bitartrate taken as 552.

(iii) Anti-5-hydroxytryptamine activity of ergot alkaloids: The effect of different concentrations of the ergot alkaloids, ergonovine, methylergonovine, ergotamine, and dihydroergotamine, on dose-response curves of 5-HT was studied in five experiments. In each experiment, separate strips obtained from the same animal were used to study the antagonism between 5-HT and different ergot alkaloids. Dose-response curves based on three graded doses were made for 5-HT. Then the curves were determined again in the presence of the ergot alkaloid being investigated. For this purpose, the concentration of the alkaloid was not maintained in the bath during whole of the experiment but the tissue was exposed to the same dose of the alkaloid only for 5 minutes before each test of the response to 5-HT. It was thus possible to demonstrate the antagonistic effect of ergonovine and methylergonovine with as low concentrations as 10^{-11} g/ml. Similarly dose-response curves for 5-HT were repeated in the presence of increasing concentrations (usually 3×10^{-11}, 10^{-10}, 3×10^{-10} g/ml) of ergonovine or methyl-

ergonovine. Higher concentrations (10^{-9} g/ml or more) of these alkaloids usually contracted the tissue, interfering with the evaluation of their antagonistic effects. With ergotamine, such interference was present even at lower concentrations.

(iv) Relationship between the degree of 5-hydroxytryptamine antagonism and the exposure time to the inhibitory substance: Gaddum et al (1955) showed that the intensity of 5-HT block produced by LSD increased with the time during which the rat uterus stayed in contact with the inhibitor. This behaviour has also been studied in the following way for BOL, ergonovine, and methylergonovine in dog urinary bladder preparations.

After the dose-response curve for 5-HT had been plotted, a predetermined concentration of the inhibitor was added to the bath. This concentration was maintained in the bath during the rest of the experiment by immediately adding fresh doses of the inhibitor after each bathing fluid change. After 5 minutes contact with the inhibitor, the effect of a test dose (A) of 5-HT was measured. From the dose-response curve plotted at the beginning of the experiment, the dose (A_o) whose effect was similar to that caused by the test dose (A) in the presence of the inhibitor, was found. The dose-ratio (A/A_o) was then calculated. Test responses with higher concentrations of 5-HT were measured at twenty minute intervals and dose-ratios calculated. The dose-ratios were then plotted against time and the curves obtained for different inhibitors.

Effect of Reserpine Pretreatment. The possibility that the ergot alkaloids may act indirectly through the release of 5-HT was tested by comparing the responses of the preparations obtained from reserpine

pretreated animals to those obtained from normal animals. Dogs were given reserpine (1 mg/kg intraperitoneally) 24 hours before the experiment. Urinary bladder strips were set up in the usual way. A preparation from a normal animal was also set up as a control. Dose-response curves for the agonists were plotted for each tissue. The sensitivity of both types of preparations in respect of their contractile responses to various agonists was compared by plotting the curves which represented the mean of experiments in this series.

C. DRUGS AND SOLUTIONS

The compounds used in this study and the sources from where they were obtained are listed below. All solutions unless otherwise specified were made weight/volume in terms of the base. Stock solutions were stored at 4°C. Concentrations mentioned in the text are the final concentrations in the bath fluid in terms of g/ml. At no time was more than 1 ml of a testing solution added to the tissue bath.

Agonists. Stock solutions 1-10 mg/ml were made in 0.01M HCl. On the morning of use, the stock solutions were diluted as required with acidified 0.9% sodium chloride solution.

Acetylcholine chloride (British Drug Houses)

l-Adrenaline bitartrate (Sterling Winthrop)

Ergonovine maleate (Sandoz)

Histamine diphosphate (Nutritional Biochemical Corporation)

5-Hydroxytryptamine creatinine sulphate (Calbiochem B Grade)

Methylergonovine maleate (Sandoz)

l-Noradrenaline bitartrate (Calbiochem)

Antagonists. Stock solutions 1 mg/ml of the salt were made in distilled water. Suitable dilutions were made daily in 0.9% sodium chloride solution.

Atropine sulphate (British Drug Houses)

Cocaine hydrochloride (British Drug Houses)

Diphenhydramine hydrochloride (Parke Davis & Company)

Dimethylphenylpiperazinium iodide (Parke Davis & Company)

Hexamethonium chloride (Burroughs Wellcome & Company)

Morphine sulphate (Ingram & Bell)

2-Bromolysergic acid diethylamide (BOL Sandoz). BOL was supplied in 1 ml ampoules containing BOL bitartrate 0.5 mg, tartaric acid 0.25 mg, and sodium chloride 0.8 mg in distilled water. Suitable dilutions were made in 0.9% NaCl solution just before use.

Dihydroergotamine methanesulphonate (Sandoz). This was supplied in 1 ml ampoules containing 1 mg of the salt. Suitable dilutions were made in acidified 0.9% NaCl solution on the morning of use.

Ergotamine tartrate (Sandoz). Stock solutions containing 1 mg/ml of the base were made in 0.01 M HCl and kept refridgerated. Dilutions when required were made in acidified 0.9% NaCl solution.

Phenoxybenzamine hydrochloride (POB) (Smith Kline & French). POB stock solutions containing 10 mg/ml were made in propylene glycol and 0.1 M HCl and stored at 4°C. Dilutions when required were made in acidified 0.9% NaCl solution.

Reserpine (Ciba Co Ltd). Stock solutions 5 mg of the base/ml of reserpine which was to be used for intraperitoneal injection, was prepared by dissolving 100 mg reserpine in a mixture of 2 ml glacial acetic

acid, 2.5 ml propylene glycol, 2.5 ml ethanol and distilled water to 20 ml volume.

Krebs-Henseleit solution. The bathing fluid for the tissues had the following composition.

Substance	Concentration g/l	Concentration mM
NaCl	6.9	118.0
KCl	0.35	4.7
$CaCl_2$	0.28	2.5
KH_2PO_4	0.16	1.1
$MgSO_4$	0.14	1.2
$NaHCO_3$	2.20	25.0
Glucose	2.00	11.0

SECTION III

RESULTS

CHAPTER 6

RESULTS

A. GUINEA-PIG ILEUM

Comparative Activity

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Dose-Ratios

Desensitization Studies

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Effect of Reserpine Pretreatment

Partial Agonists

Antagonistic Activity of Ergotamine and Dihydroergotamine

B. DOG URINARY BLADDER

Sensitivity to Drugs

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Determination of pA_2 with BOL

Anti-5-Hydroxytryptamine Activity of Ergot Alkaloids

Relationship between the Degree of 5-HT Antagonism and the Exposure Time to the Inhibitory Substances

Effect of Reserpine Pretreatment

Of the four alkaloids tested on guinea-pig ileum and dog bladder, only ergonovine and methylergonovine produced definite contractions of both tissues. Ergotamine and dihydroergotamine, even in very high concentrations, had no stimulatory effect in guinea-pig ileum. However, these peptide alkaloids produced sluggish but definite contractions of the dog urinary bladder strips.

Results of the detailed investigations regarding the type of receptors involved in the effects of the ergot alkaloids on these preparations are described below.

A. GUINEA-PIG ILEUM

Guinea-pig ileum is normally quiescent and shows little rhythmic activity. When ergonovine or methylergonovine was added to the bath, the rhythmic contractions usually stopped and the tissue showed a tonic contraction reaching the maximum in 60-90 seconds. The muscle relaxed and recovered its original length in less than three minutes even if the drug was not washed out. The pattern of contraction was thus similar to that due to 5-HT, although the tissues take longer to reach the peak contraction. The height of contraction due to a given dose did not vary markedly from tissue to tissue. No evidence of tachyphylaxis was observed, since repeated doses of the ergot alkaloids, given at 15 min intervals, showed no diminution of response.

Comparative Activity. Fig. 2 shows dose-response curves for the agonists, acetylcholine, histamine, 5-HT, ergonovine, and methylergonovine. By comparing the threshold doses of the agonists, the ergot alkaloids are found to be less potent than the standard agonists, their relative activities being 1/10 of 5-HT, 1/100 of histamine, and 1/300 of

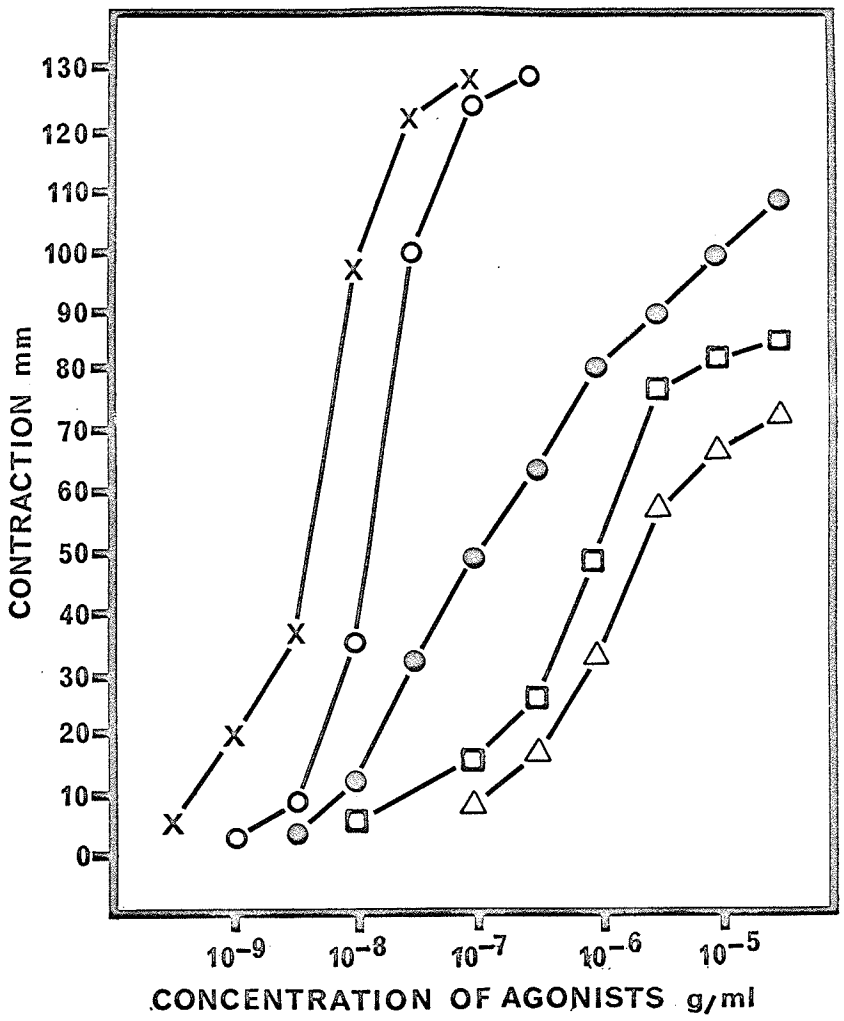

Fig. 2. LOG DOSE-RESPONSE CURVES OF GUINEA-PIG ILEUM TO AGONISTS.

Acetylcholine x – x , histamine O – O, 5-hydroxytryptamine ◉ – ◉, methylergonovine □ – □, ergonovine △ – △.

acetylcholine. The sensitivity of the guinea-pig ileum (threshold concentration) to ergonovine and methylergonovine is similar, although the maximum contraction of the tissue obtained with methylergonovine is more than that with ergonovine.

In all subsequent experiments, the doses of the various agonists given were selected from doses usually giving between 30 and 70% of the maximal contraction for the agonist, i.e., from the straight parts of the dose-response curves as shown in Fig. 2. Usually three graded doses were employed.

Antagonism Studies. Preliminary studies indicated that the ergot alkaloids might be acting through 5-HT receptors in guinea-pig ileum. Gaddum and Picarelli (1957) suggested the presence of two kinds of receptors sensitive to 5-HT in this tissue, i.e., 'M' receptors which could be blocked by atropine, cocaine, or morphine and 'D' receptors blocked by Dibenzyline, lysergic acid diethylamide or dihydroergotamine. The effect of various antagonists was therefore studied to obtain information about the contribution of the two kinds of receptors towards the pharmacological actions of the ergot alkaloids.

Dose-response curves for the agonists, usually based on three graded doses, were obtained in each tissue as control responses. Thereafter, responses to the same doses of the agonists were obtained in the presence of the antagonists. In some cases where the antagonists almost abolished the responses of the agonists, higher concentrations of the agonists were also tested to see whether the block with the particular concentration of the antagonist was surmountable or not.

The effect of the antagonists has been studied by observing the

degree of the displacement of the curves of the various agonists and the effect of the antagonists has been measured in terms of dose-ratio also.

Effect of Atropine. In a series of 8 experiments atropine in a concentration of 10^{-8} g/ml, which completely blocked responses to acetylcholine, had hardly any effect on the responses to the ergot alkaloids. The contractions due to 5-HT were much inhibited but the responses to histamine were almost unaffected. Fig. 3 illustrates the results obtained in a typical experiment.

Effect of Cocaine. When the ileum was treated with cocaine (5×10^{-8} g/ml), contractions due to acetylcholine and histamine remained unaffected. In case of ergot alkaloids, there was slight displacement of the curves to the right (Fig. 4). Responses to 5-HT were markedly inhibited, an effect consistent with the view that 5-HT acts at some point in the intramural nervous system.

Effect of Morphine. A concentration of 5×10^{-7} g/ml was used for studying the antagonism by morphine of the stimulatory responses of the agonists. At this concentration, morphine only slightly inhibited the tone of guinea-pig ileum segments. Responses to 5-HT were markedly inhibited by morphine whereas those to acetylcholine and histamine were affected only a little. As regards the ergot alkaloids, morphine inhibited their responses to a variable extent. Only slight inhibition was seen in 7 experiments. Fig. 5 shows the results of a representative experiment in this series. In another 3 experiments a more pronounced inhibition was observed. However, the inhibition of the responses to ergot alkaloids was never as marked as the inhibition of the responses to 5-HT.

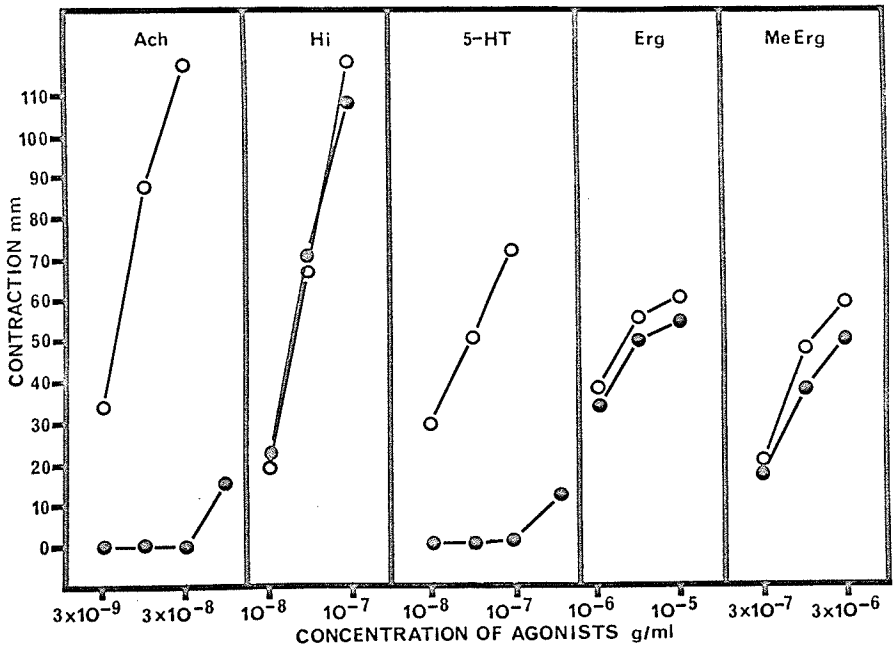

Fig. 3. EFFECT OF ATROPINE ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before atropine O — O, log dose-response curves with atropine (10^{-8} g/ml) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

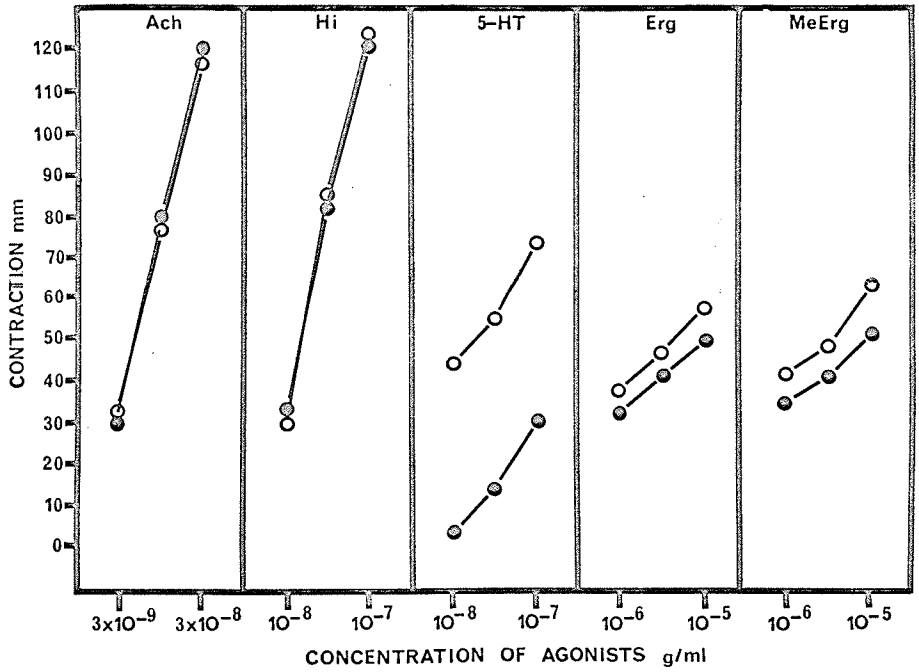

Fig. 4. EFFECT OF COCAINE ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before cocaine O — O, log dose-response curves with cocaine ($5x10^{-8}$ g/ml) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

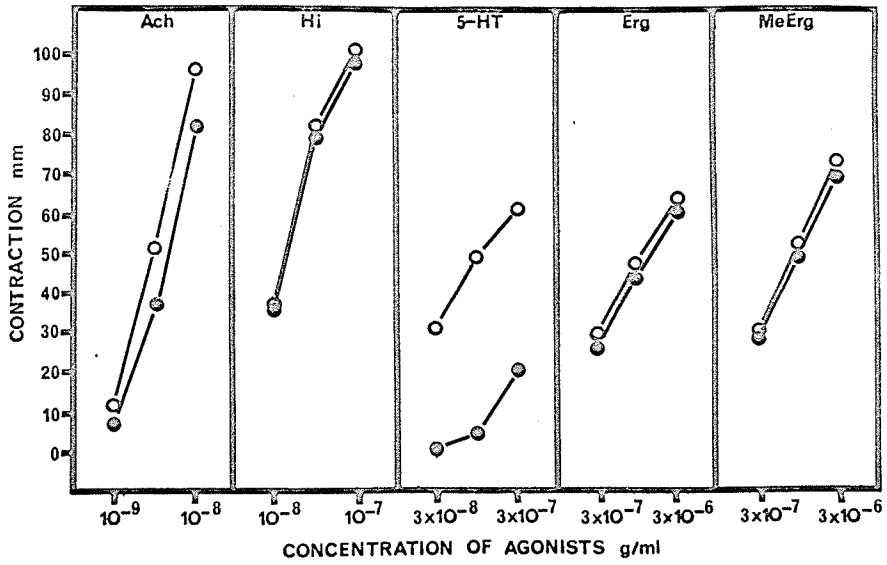

Fig. 5. EFFECT OF MORPHINE ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before morphine ○—○, log dose-response curves with morphine 5×10^{-7} ●—●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

Effect of Bromolysergic Acid Diethylamide. The tissues were exposed to BOL for only five minutes before each test. Used in this way, BOL itself had virtually no stimulatory effect; there was only occasionally a slight increase in the basal tone of the segments. Further, BOL had no cumulative effect used in this way. BOL in a concentration of 5×10^{-7} g/ml reduced the responses to 5-HT and methylergonovine but had no effect on histamine responses. In case of acetylcholine, there was slight potentiation especially at lower doses. Effect on ergonovine responses was variable. Higher concentrations of BOL (5×10^{-6} g/ml) almost completely abolished the responses to 5-HT but had little effect on responses to acetylcholine or histamine. Responses to both ergonovine and methylergonovine were markedly inhibited (Fig. 6). These results suggested a common receptor site for 5-HT and the ergot alkaloids.

Effect of Phenoxybenzamine. Responses of the ileum to the agonists were measured before and after a single exposure of 5 minutes to phenoxybenzamine. POB (1×10^{-7} g/ml) almost completely abolished responses to the ergot alkaloids (Fig. 7). There was slight displacement of the curves for other agonists to the right. However, contractions due to 5-HT were inhibited to approximately the same extent as those due to acetylcholine and histamine.

Effect of Phentolamine. Phentolamine was applied to ileum in concentrations of 10^{-8} to 10^{-6} g/ml. At lower concentrations, phentolamine did not affect the contractions produced by any of the agonists. At higher concentrations, it slightly depressed the responses of all the agonists equally.

Effect of Hexamethonium. When the preparation was treated with the competitive ganglion blocking agent, hexamethonium (5×10^{-6} g/ml),

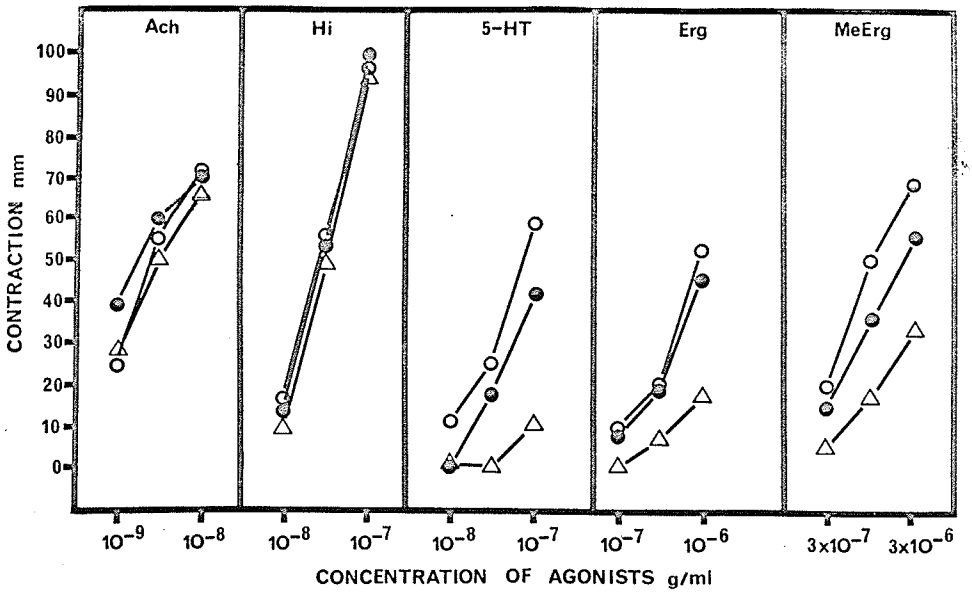

Fig. 6. EFFECT OF BOL ON RESPONSES OF GUINEA-PIG ILEUM

Log dose-response curves before BOL ○ — ○, log dose-response curves with BOL ($5x10^{-7}$g/ml) ● — ●, log dose-response curves with BOL ($5x10^{-6}$g/ml) △ — △.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

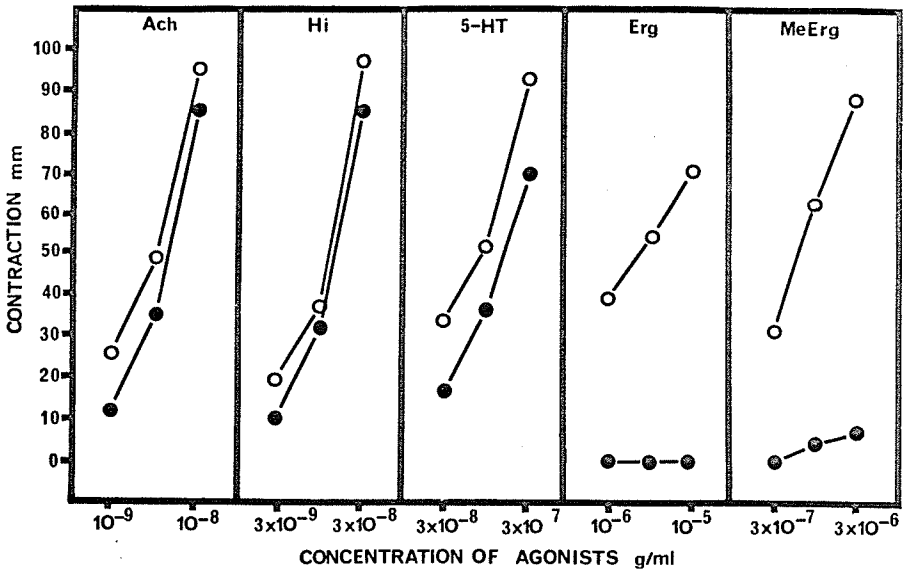

Fig. 7. EFFECT OF PHENOXYBENZAMINE ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before phenoxybenzamine O — O, log dose-response curves after phenoxybenzamine (10^{-7}g/ml) ● — ● .

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

the responses to the various agonists were not affected to any significant extent.

Effect of Dimethylphenylpiperazinium. DMPP in high concentrations is believed to block ganglia by depolarizing the ganglion cell (Ling, 1959). DMPP (5×10^{-6} g/ml) only slightly depressed responses to acetylcholine and histamine but markedly inhibited responses to 5-HT. Responses to the ergot alkaloids were also depressed but much less than were responses to 5-HT. The block produced by DMPP was reversed rapidly on washing. Fig. 8 shows the result of a typical experiment.

Effect of Diphenhydramine. Fig. 9 shows the result of an experiment in which the ileum was treated with an antihistaminic, diphenhydramine (10^{-7} g/ml). The responses to histamine were almost completely abolished but the responses to the other agonists were unaltered, showing that the contractile response of the guinea-pig ileum to the ergot alkaloids was not through release of histamine.

Dose-Ratios. Although the dose-response curves in the antagonism experiments were incomplete in that the maximum effect was never reached, an approximate calculation of the ratio of doses of equal potency in the presence and absence of the antagonists was made. Whenever possible the dose-ratios were determined from the values at the centre of the dose-response curves. In some cases, the slope of the curve was less steep after the addition of the antagonist. Kosterlitz and Robinson (1958) compared the smallest dose before with the largest dose after the addition of the antagonist in order to estimate approximate dose-ratios in such cases where the slope of the curve was less steep. The same method has been used in the present study. For this reason, the estimates

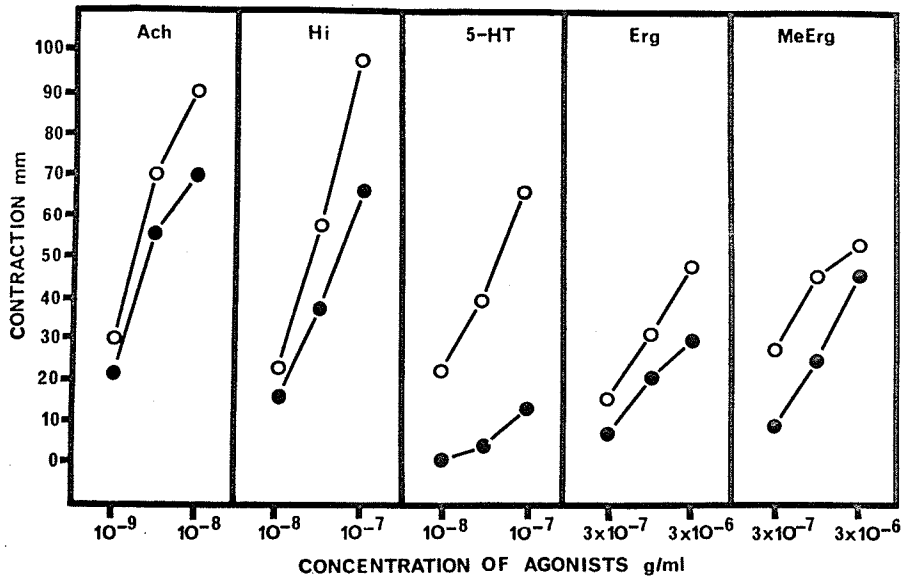

Fig. 8. EFFECT OF DMPP ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before DMPP ○ — ○, log dose-response curves with DMPP ($5x10^{-6}$ g/ml) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

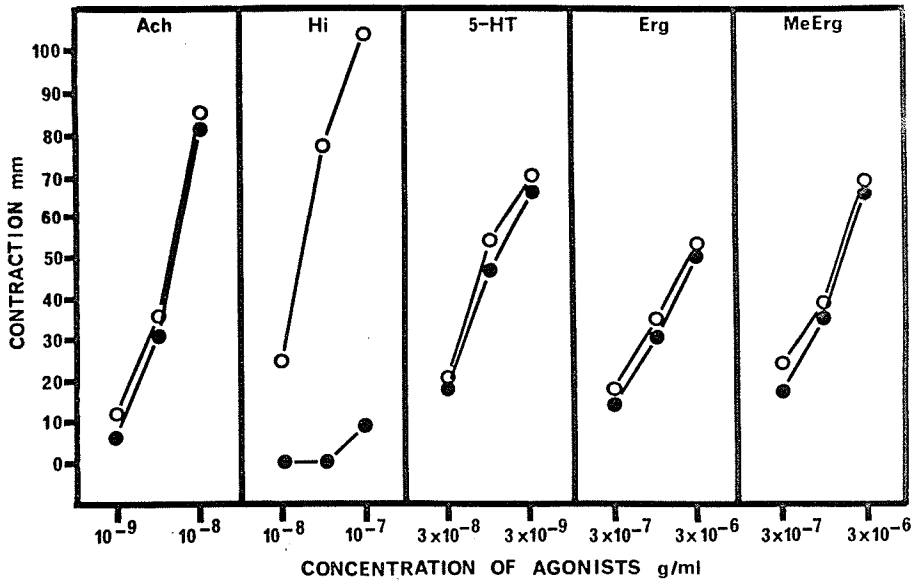

Fig. 9. EFFECT OF DIPHENHYDRAMINE ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before diphenhydramine ○ – ○, log dose-response curves with diphenhydramine (10^{-7} g/ml) ● – ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

given can be used as a rough guide only. Table I summarizes the estimates of dose-ratios in respect of the various antagonists.

Desensitization Studies. The concentration of each desensitizing agent which would render the tissue insensitive to the desensitizing agent without affecting the sensitivity of acetylcholine or histamine was determined in preliminary experiments. These were found to be 2 x 10^{-6} g/ml of 5-HT and 10^{-4} g/ml of ergonovine or methylergonovine.

Dose-response curves for each of the agonists (acetylcholine, histamine, 5-HT, ergonovine, and methylergonovine) were obtained in four segments of the ileum. Desensitization with 2 x 10^{-6} g/ml of 5-HT, 10^{-4} g/ml of ergonovine, and 10^{-4} g/ml of methylergonovine was carried out as described on page 47 , with one of the desensitizing agents in each of three segments. The fourth segment, to which no desensitizing agent was added, was used as control. The responses to the same doses of the agonists as given for the control curves were then determined in the presence of the desensitizing agent. Fig. 10 shows the dose-response curves obtained in one such experiment. Responses to acetylcholine and histamine were not markedly modified by any of the desensitizing agents while the stimulatory effects of 5-HT, ergonovine, and methylergonovine were almost totally abolished by desensitization not only with 5-HT but also with either of the ergot alkaloids.

Results of a series of such experiments are summarized in Table II. The figures in the table represent the remaining response expressed as a percentage of the control response at each dose level used for the various agonists. Each of the desensitizing agents affected the responses of the guinea-pig ileum to the various agonists in a similar fashion. The residual responses to acetylcholine and histamine were slightly de-

TABLE 1

RATIOS OF DOSES OF ACETYLCHOLINE, HISTAMINE, 5-HT, ERGONOVINE, AND METHYLERGONOVINE CAUSING EQUAL CONTRACTIONS OF GUINEA-PIG ILEUM IN THE PRESENCE AND ABSENCE OF ANTAGONISTS

Antagonists (g/ml)	DOSE RATIOS OF AGONISTS MEAN (RANGE)				
	Acetylcholine	Histamine	5-HT	Ergonovine	Methylgonovine
Atropine 10^{-8}	25.3 (12.6-44.6)	1.8 (1.0-3.2)	27.9 (10.0-56.2)	1.3 (1.0-2.2)	1.1 (1.0-1.6)
Cocaine 10^{-8}	1.0 (.8-1.4)	1.1 (1.0-1.4)	19.5 (3.9-79.6)	1.5 (.7-2.5)	1.4 (.6-3.2)
Morphine 5×10^{-7}	1.8 (1.0-3.2)	1.3 (1.0-1.99)	(3.2-177.8)	2.3 (1.0-10.0)	1.7 (1.0-3.2)
POB 10^{-7}	2.6 (1.7-3.5)	2.8 (1.7-3.5)	2.6 (1.4-4.4)		
BOL 5×10^{-7}	0.99 (0.45-1.25)	1.0	2.1 (0.63-5.6)	1.47 (0.77-1.77)	4.4 (2.8-6.3)
DMPP 5×10^{-6}	1.6 (1.4-1.7)	1.8 (1.2-2.5)	9.4 (1.6-22.3)	2.12 (1.0-5.0)	2.18 (1.0-3.9)

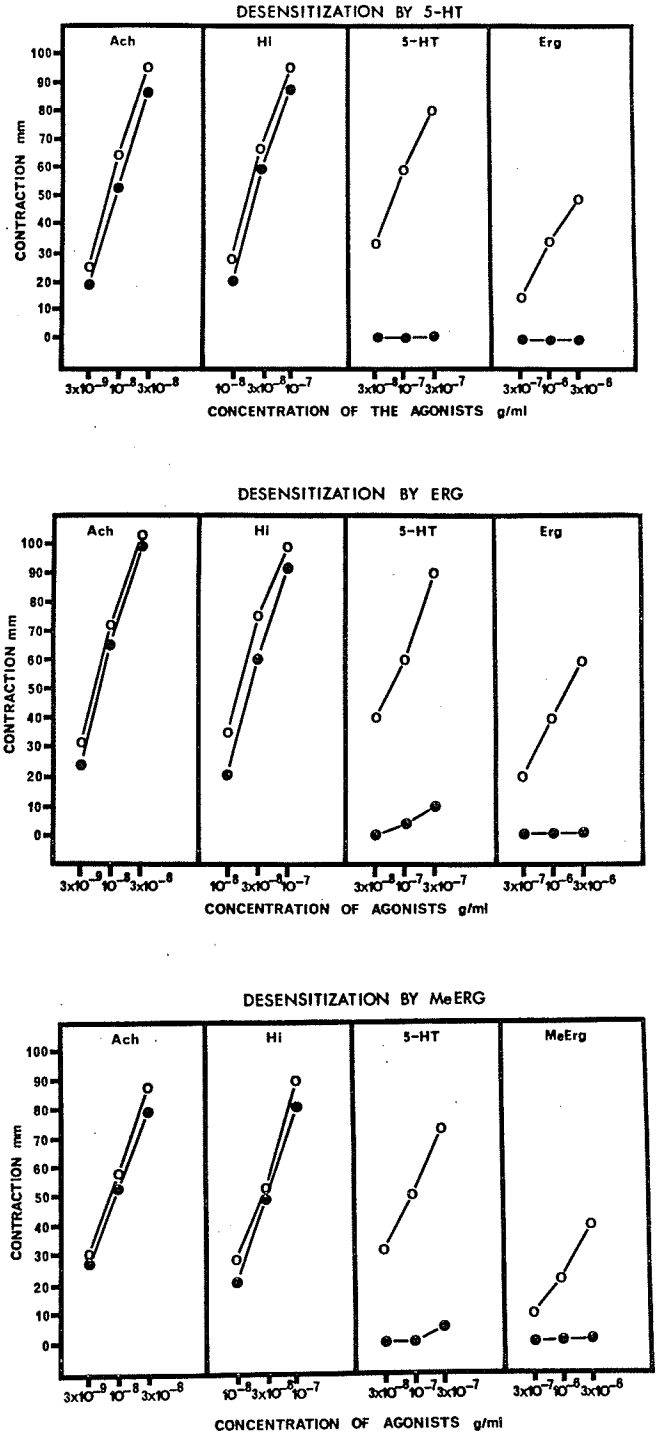

Fig. 10. EFFECT OF DESENSITIZATION TO RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before desensitization O – O , log dose-response curves after desensitization ● – ● . Desensitizing dose of 5-hydroxytryptamine $1x10^{-6}$g/ml, desensitizing dose of ergonovine $1x10^{-4}$g/ml, desensitizing dose of methylergonovine $1x10^{-4}$g/ml.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

TABLE II

RESPONSES TO VARIOUS AGONISTS IN GUINEA-PIG ILEUM AFTER DESENSITIZATION.
RESIDUAL RESPONSES EXPRESSED AS PERCENT OF CONTROL

DESENSITIZING AGENT	AGONISTS	CONCENTRATION g/ml						
		$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	$3x10^{-7}$	10^{-6}	$3x10^{-6}$
5-HT $2x10^{-6}$ g/ml	Acetylcholine	69	75	89	90			
	Histamine		64	69	79			
	5-HT		0	0	0	3		
	Ergonovine					0	0	0
	Methylergonovine					0	0	0
Ergonovine 10^{-4}	Acetylcholine	72	89	89	100			
	Histamine		66	75				
	5-HT		0	0	7	9		
	Ergonovine					0	0	0
	Methylergonovine					0	0	0
Methyl-ergonovine 10^{-4}	Acetylcholine	71	79	86	98			
	Histamine							
	5-HT		0	1	4	6		
	Ergonovine					0	2	3
	Methylergonovine					0	0	1

creased and the responses to 5-HT, ergonovine, and methylergonovine virtually abolished at all dose levels. The decreases were approximately the same with each of the desensitizing agents. In all experiments, responses to histamine were slightly more affected than those to acetylcholine. The average response to the highest tested dose of 5-HT was only 6% of the control response, when the preparations were desensitized with methylergonovine. Corresponding values were 9% and 3% after desensitization with ergonovine and 5-HT respectively. Responses to ergonovine and methylergonovine were completely abolished at all dose levels when the preparations were desensitized with 5-HT or ergonovine. When methylergonovine was used as desensitizing agent, responses to ergonovine and methylergonovine, at the highest test level, were only 3% and 1% respectively. It is evident that the desensitization procedure leads to a clear-cut differentiation between receptors for acetylcholine and histamine on the one hand and receptors for the other agonists on the other. Cross-desensitization between 5-HT and the ergot alkaloids support the view that both ergonovine and methylergonovine act through 5-HT receptors in guinea-pig ileum.

Effects of Cold Storage. To investigate if the contractile response of the guinea-pig ileum to the ergot alkaloids is mediated, wholly or partly, through the excitation of the intrinsic network of nerves, responses to various agonists were compared before and after storage of ileum at 4°C for 24-72 hours, a procedure which preferentially inactivates the nervous activity.

The responses to acetylcholine and histamine were not modified to a great extent by cold storage of the tissues. On the other hand, responses to 5-HT were markedly depressed. These results agree with the

conclusions arrived at by various authors (Emmelin and Feldberg, 1947; Feldberg, 1951; Ambache and Lessin, 1955; Innes et al., 1957; Kosterlitz and Robinson, 1958; Day and Vane, 1963) that acetylcholine and histamine act mainly through the direct excitation of smooth muscle whereas 5-HT acts through receptors in nervous tissue.

In 6 out of 8 experiments, responses to the ergot alkaloids were depressed to a variable extent at different dose levels. Responses to low doses were only slightly depressed. At high doses, the depression was more marked. In no case was the depression as marked as that of 5-HT. Fig. 11 shows the dose-response curves of the various agonists in one such experiment.

In one experiment, responses to the ergot alkaloids was depressed to the same extent as those of 5-HT. However, in this experiment responses to acetylcholine and histamine were also markedly depressed. Presumably these observations represent changes in the sensitivity or contractility of the muscle fibres. In another experiment in which the ileum was stored for 24 hours, responses to the ergot alkaloids were hardly affected. In this experiment, DMPP (5×10^{-6} g/ml), a ganglionic stimulant, produced a contractile response, showing that cold storage had not successfully abolished the function of the nervous tissue.

Effect of Reserpine Pretreatment. The effects of ergonovine and methylergonovine were tested on segments of guinea-pig ileum depleted of 5-HT by reserpine to find out if any component of the contractile response to the ergot alkaloids was due to release of endogenous 5-HT.

Doses of ergonovine and methylergonovine which caused contrac-

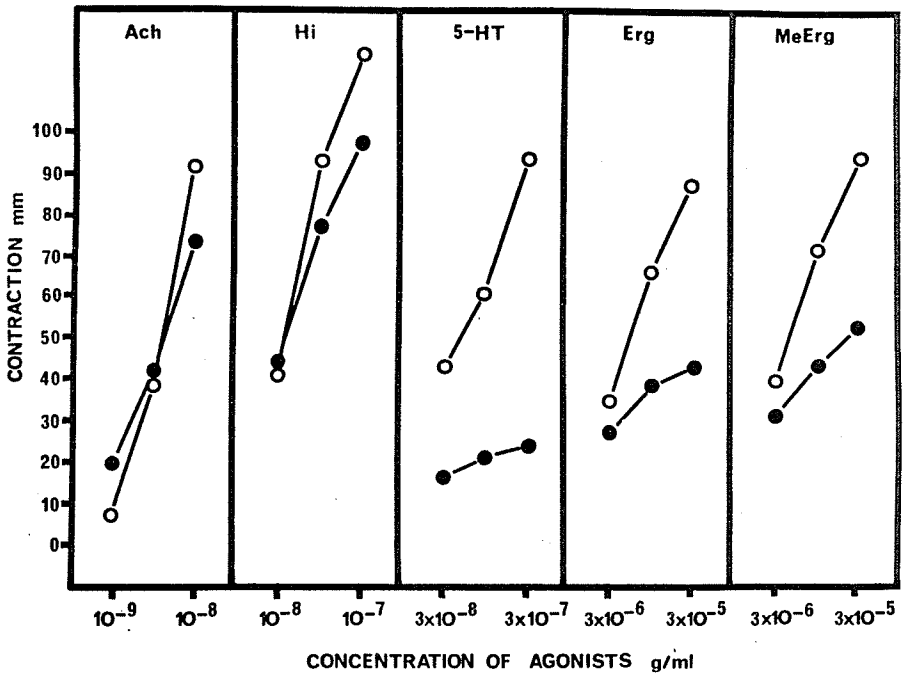

Fig. 11. EFFECT OF COLD STORAGE ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves of fresh ileum ○—○, log dose-response curves of ileum stored at 4°C for 48 hours ●—●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxy-tryptamine, 5-HT; Ergonovine, Erg; Methylergono-vine, MeErg.

tions in ileal segments from normal animals produced contractions of the segments from reserpine pretreated animals, showing that the contractile response to the ergot alkaloids cannot be attributed to the release of endogenous 5-HT.

When the dose-response curves for the various agonists in reserpine pretreated animals were compared to those in untreated animals, a marked increase in the sensitivity of the preparations from reserpine pretreated animals was found. This increase in sensitivity was, however, greater for acetylcholine, histamine or 5-HT than for the ergot alkaloids.

Fig. 12 shows the mean results of eight experiments in which the contractile responses to the various agonists were recorded in preparations taken from reserpine pretreated as well as untreated animals.

Partial Agonists. As indicated earlier, both ergonovine and methylergonovine possess appreciable agonistic activity of their own and also antagonistic activity against 5-HT. The maximal contractile responses to ergonovine and methylergonovine are less than the maximum response to 5-HT (Fig. 2). These observations suggest that the ergot alkaloids may be partial agonists on 5-HT receptors. The combined action of 5-HT with ergonovine or methylergonovine on guinea-pig ileum was therefore studied to determine whether these drugs occupied the same receptor sites.

Dose-response curves for ergonovine alone and also combined with 10^{-8}, 3×10^{-8}, 10^{-7}, 3×10^{-7} g/ml of 5-HT were plotted. This resulted in a characteristic family of dose-response curves, the study of which reveals a dualism in action of ergonovine. There is synergism or antagonism depending on the concentrations of the drugs. With low concentration of 5-HT, ergonovine acts as a synergist. When high concentrations of 5-HT are combined with graded doses of ergonovine, there is

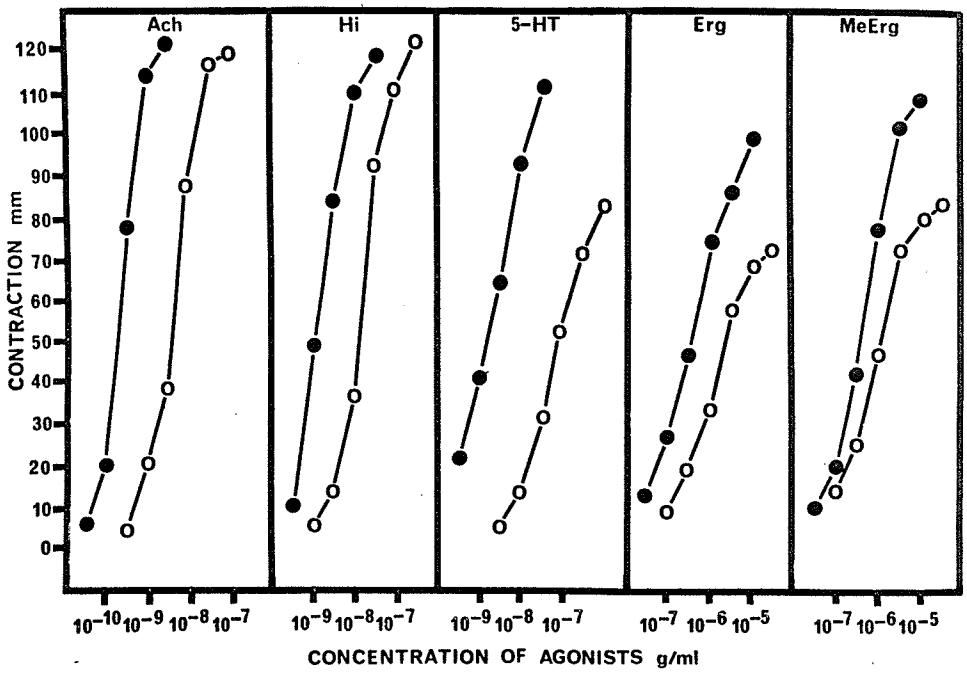

Fig. 12. EFFECT OF RESERPINE PRETREATMENT ON RESPONSES OF GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves of untreated ileum O — O, log dose-response curves of reserpine pretreated ileum (1 mg/kg/24 hours) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

increasing antagonism at the higher dose-levels of ergonovine. However, the highest dose of ergonovine, independent of the concentration of 5-HT combined with it, always results finally in the same response, suggesting that ergonovine given in adequate concentration finally occupies all 5-HT receptors.

Fig. 13 shows the dose-response curves in one experiment with ergonovine as the partial agonist. Dose-response curves in case of methylergonovine were affected in a similar manner.

Antagonistic Activity of Ergotamine and Dihydroergotamine. Ergotamine and dihydroergotamine had no stimulatory effect in guinea-pig ileum, even in the highest tested dose of 10^{-5} g/ml. The antagonistic activity of these alkaloids against various agonists including sympathomimetic amines, was studied in terminal as well as nonterminal segments.

Fig. 14 shows the effect of ergotamine 10^{-7} g/ml on the dose-response curves of the various agonists when tested in a terminal segment of the ileum. Ergotamine completely abolished stimulatory response to noradrenaline, an α adrenergic activity. It did not antagonize responses to acetylcholine; rather there was some potentiation at lower dose levels. Responses to 5-HT, ergonovine, and methylergonovine were all inhibited. Inhibition of 5-HT was much more pronounced than inhibition of ergonovine or methylergonovine.

In nonterminal segments, the effect of graded doses of noradrenaline and isoprenaline on the responses of coaxially stimulated ileum was measured as percentage decrease in the height of contraction, from which dose-response curves for noradrenaline and isoprenaline were plotted. The effect of the same doses of noradrenaline and isoprenaline was then measured in the presence of the blocking agents. The dose-re-

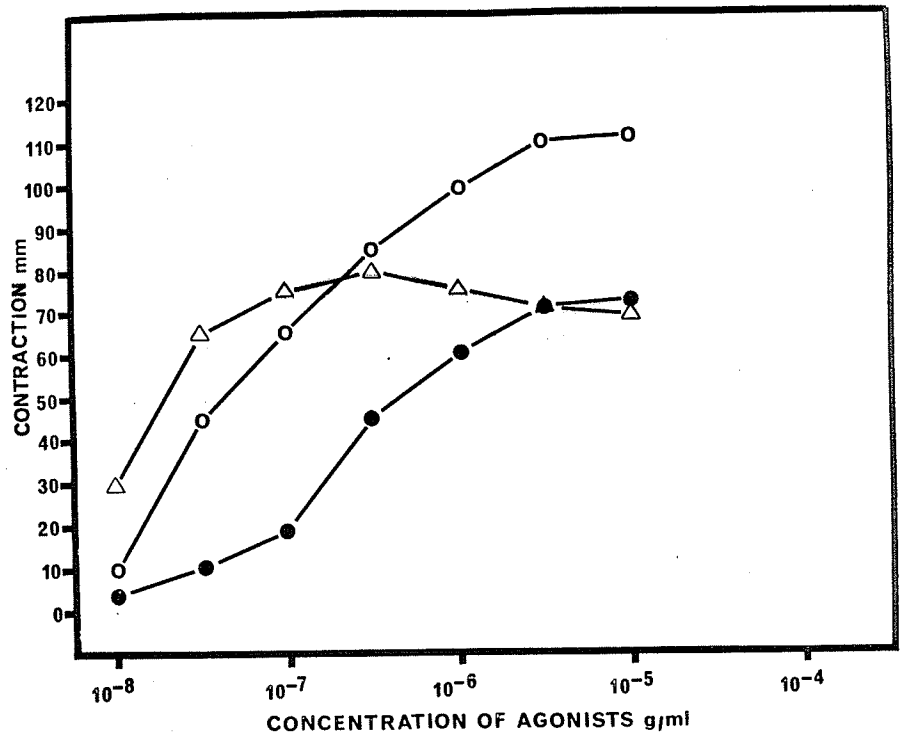

Fig. 13. RESPONSES OF GUINEA-PIG ILEUM TO 5-HYDROXYTRYPTAMINE ALONE, O — O , TO ERGONOVINE ALONE, ● — ●, AND TO 5-HYDROXYTRYPTAMINE AND ERGONOVINE GIVEN TOGETHER; Δ — Δ.

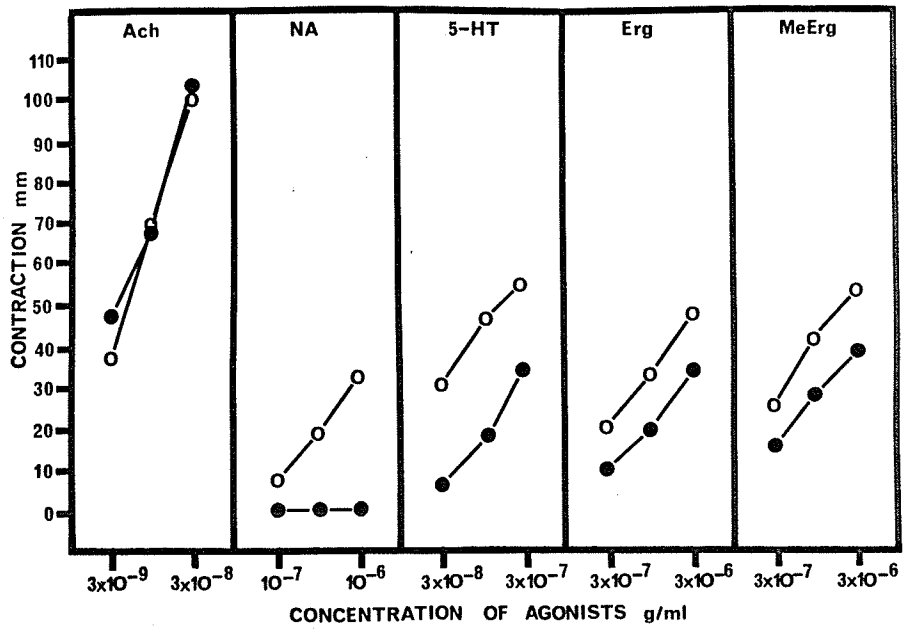

Fig. 14. EFFECT OF ERGOTAMINE ON RESPONSES OF TERMINAL GUINEA-PIG ILEUM TO AGONISTS.

Log dose-response curves before ergotamine O—O, log dose-response curves with ergotamine (10^{-7}g/ml) ●—●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

sponse curves were constructed and compared to the control curves.

In one set of experiments, the blocking effect of ergotamine or dihydroergotamine was studied first. These ergot alkaloids had no effect on the action of isoprenaline but partially blocked the inhibitory action of noradrenaline. There was a shift of the dose-response curve by almost 1/2 log unit (Fig. 15). When pronethalol was added in addition to ergotamine or dihydroergotamine, the inhibitory actions of both noradrenaline and isoprenaline were completely blocked.

In another set of experiments, the order of adding the blocking agents to the organ bath was reversed. In these experiments, the effect of pronethalol was studied first. Pronethalol completely blocked the inhibitory action of isoprenaline but only partially blocked the action of noradrenaline. There was a shift of the dose-response curve by about 1/4 log unit (Fig. 16). When ergotamine or dihydroergotamine was added in addition to pronethalol, complete blockade of the inhibitory action of noradrenaline also occurred.

B. DOG URINARY BLADDER

Most preparations exhibited some degree of spontaneous activity which, however, did not interfere with the measurement of the responses to various agonists. The contractions produced by agonists were sluggish, taking up to 4 minutes to reach their maximum. The time required to produce peak effect in the case of ergonovine and methylergonovine was more than the standard agonists, acetylcholine, histamine, and 5-HT. However, with all agonists, the pattern of contraction varied with the dose. With low drug concentrations, the maximum was reached in stages, with the record of drug-induced contraction interrupted by con-

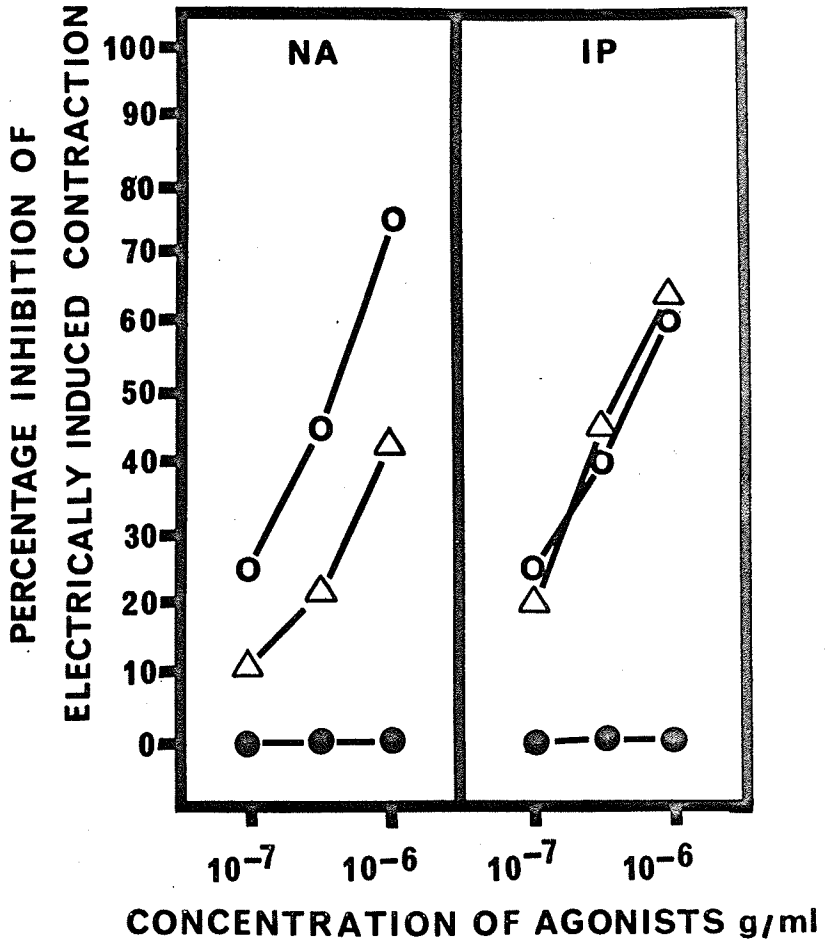

Fig. 15. INFLUENCE OF DIHYDROERGOTAMINE AND PRONETHALOL ON THE INHIBITORY ACTIONS OF NORADRENALINE AND ISOPRENALINE IN GUINEA-PIG ILEUM.

Log dose-response curves indicate the degree of inhibition due to catecholamines of contractions induced by supramaximal coaxial stimulation at 1 stimulus/10 sec. Control O – O ; with dihydroergotamine ($3x10^{-6}$g/ml) Δ – Δ ; (increase to $1x10^{-5}$g/ml did not decrease inhibition); with both dihydroergotamine ($3x10^{-6}$g/ml) and pronethalol ($1x10^{-6}$g/ml) ● – ● .

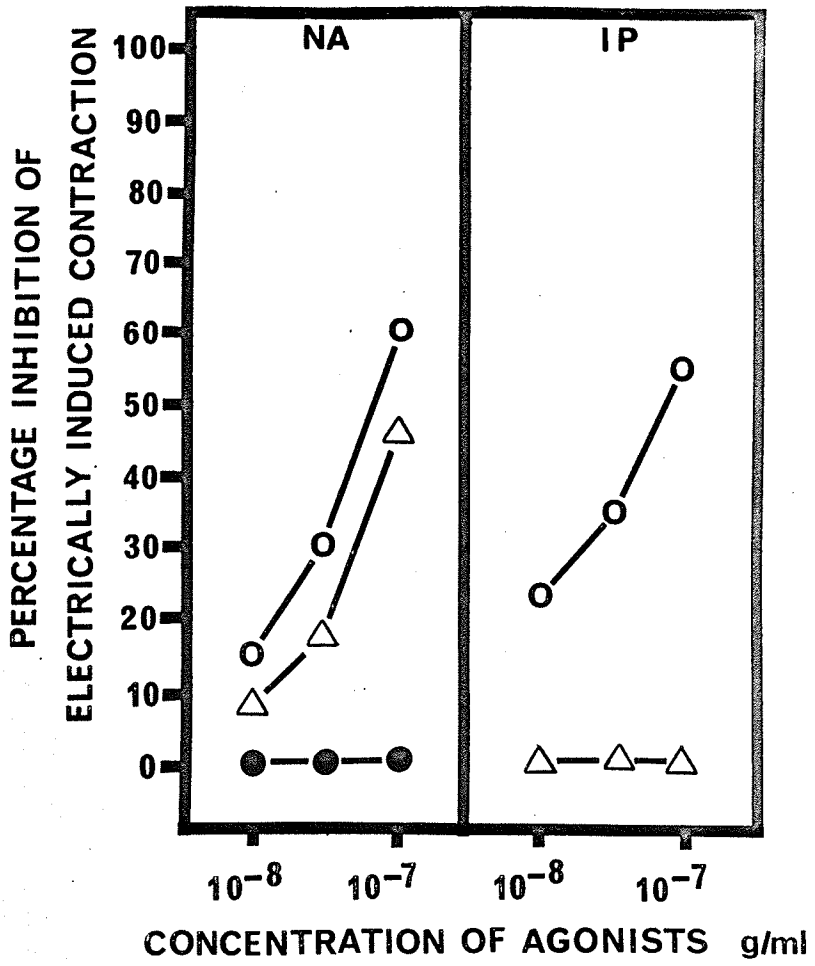

Fig. 16. INFLUENCE OF PRONETHALOL AND ERGOTAMINE ON THE INHIBITORY ACTIONS OF NORADRENALINE AND ISOPRENALINE IN GUINEA-PIG ILEUM.

Log dose-response curves indicate the degree of inhibition due to catecholamines of contractions induced by supramaximal coaxial stimulation at 1 stimulus/10 sec. Control O – O ; with pronethalol ($3x10^{-7}$g/ml) Δ – Δ ; (increase to $1x10^{-6}$g/ml did not decrease inhibition); with both pronethalol ($3x10^{-7}$g/ml) and ergotamine ($3x10^{-7}$ g/ml) ● – ● .

tinued spontaneous activity. With high concentrations, spontaneous activity was almost abolished and the tissue showed a tonic contraction. About 80% of the contraction was over in about 2 minutes in such cases. The relaxation of the muscle, after washing out the agonist, was rather slow, particularly after high doses of ergonovine and methylergonovine (10^{-8} g/ml), when return to the former base line took almost 20 minutes. No tachyphylaxis occurred if the interval between test doses was at least 30 minutes.

As already indicated in the Methods Section strips of dog bladder were cut either transversely or vertically, i.e., either at right angles or parallel to the vertical axis of the bladder, respectively. In preliminary experiments, about 10% of the transverse strips responded to noradrenaline by contraction. This response of the transverse strips was not investigated any further. As vertical strips were consistently relaxed by noradrenaline, all subsequent experiments were done with vertical strips.

Sensitivity to Drugs. Isolated strips of dog urinary bladder are quite sensitive to ergonovine and methylergonovine. Concentrations of 3×10^{-9} g/ml of either alkaloid produces contractions generally of 25 to 40 mm in strips of average sensitivity. Fig. 17 shows full dose-response curves for acetylcholine, histamine, and 5-HT. Sensitivity of this preparation to acetylcholine and histamine is about 1/30th and 1/15th respectively of the sensitivity to 5-HT. Full dose-response curves for ergonovine and methylergonovine were not plotted because the strips did not relax within a reasonable time (180 min) after higher doses of these alkaloids. Hence exact comparison between the ergot alka-

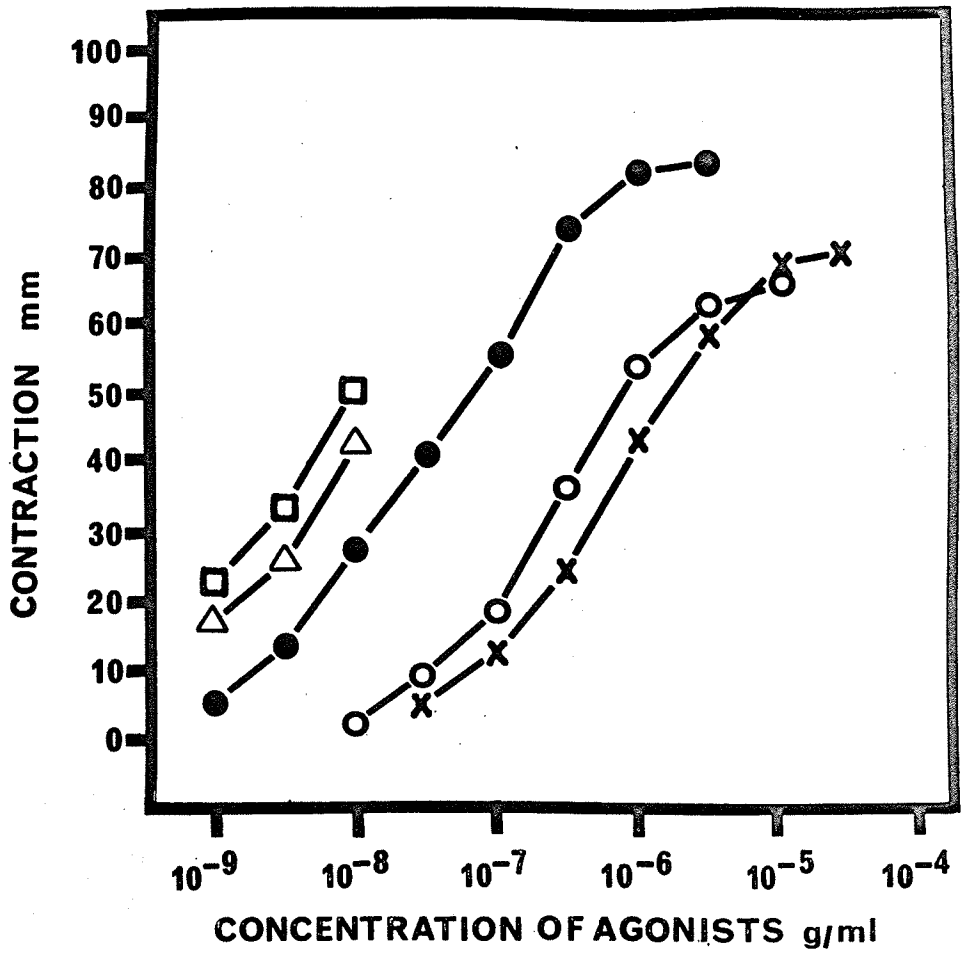

Fig. 17. LOG DOSE-RESPONSE CURVES OF DOG URINARY BLADDER TO AGONISTS.

Methylergonovine □ – □ , ergonovine Δ – Δ, 5-hydroxytryptamine ● – ● , histamine O–O, acetylcholine x – x.

loids and other agonists cannot be made.

Ergotamine and dihydroergotamine did not manifest any agonistic effect in concentrations lower than 10^{-9} g/ml. Contractile responses of the strips to higher concentrations of these alkaloids were extremely slow to develop. There was a gradual increase in the tone of the tissues and it took almost 15 minutes to reach the peak effect. It took even longer for the tissue to return to its former baseline after repeated washings. Because of this, it was difficult to plot dose-response curves or to do detailed studies of the agonistic properties of ergotamine or dihydroergotamine. Therefore only the antagonistic effects of these alkaloids were studied.

Sympathomimetic amines consistently relaxed the vertical strips. The response was dose-dependent. Prior exposure of the strips to pronethalol (3×10^{-6} g/ml) blocked the inhibitory response to noradrenaline. Higher concentrations of pronethalol (3×10^{-5} g/ml) reversed the inhibitory response to noradrenaline (3×10^{-6} g/ml) into stimulatory responses in 2 out of 6 experiments. This observation along with the stimulatory response of 10% of the transverse strips to noradrenaline, as already described, suggested the existence of adrenergic α receptors in addition to the predominant β receptors.

Antagonism Studies. The effect of the antagonists, atropine, morphine, BOL, phenoxybenzamine, phentolamine, and diphenhydramine was studied on the responses of urinary bladder strips to various agonists. At least six experiments were done for each antagonist. Dose-response curves of the agonists before and after exposure of the strips to the antagonists were compared. Results obtained in all experiments with a particular antagonist were consistent.

BOL was applied to the strips in concentrations of 10^{-9} to 10^{-7} g/ml. Responses to 5-HT, ergonovine, and methylergonovine were reduced to approximately 15-20% of control responses at a concentration of 10^{-9} g/ml. Higher concentrations completely abolished the responses to 5-HT and the ergot alkaloids but had little effect on responses to acetylcholine or histamine. These results suggested a common receptor site for 5-HT, ergonovine, and methylergonovine. Fig. 18 shows the dose-response curves of the agonists obtained in an experiment in which the effect of BOL (10^{-8} g/ml) was studied.

Phenoxybenzamine in concentrations less than 10^{-7} g/ml showed little or no antagonism to any of the agonists. A single exposure of urinary bladder strips to phenoxybenzamine (10^{-7} g/ml) for five minutes markedly inhibited the responses to 5-HT, ergonovine, and methylergonovine as compared to control responses. Responses to acetylcholine and histamine were also inhibited to the same extent. Fig. 19 shows the dose-response curves of various agonists before and after exposure of a strip to phenoxybenzamine (10^{-7} g/ml).

The effect of phentolamine was also studied. Phentolamine in concentrations less than 10^{-7} g/ml showed little or no antagonism to any of the agonists. Phentolamine (10^{-7} g/ml) slightly depressed the responses of all the agonists equally, which seems to be an unspecific effect. This observation is in conformity with above mentioned effect of phenoxybenzamine and therefore excludes the possibility of any adrenergic component in the contractile response of dog urinary bladder to ergonovine or methylergonovine. The lack of specific blockade by phentolamine or phenoxybenzamine suggest that the ergots do not act on α adren-

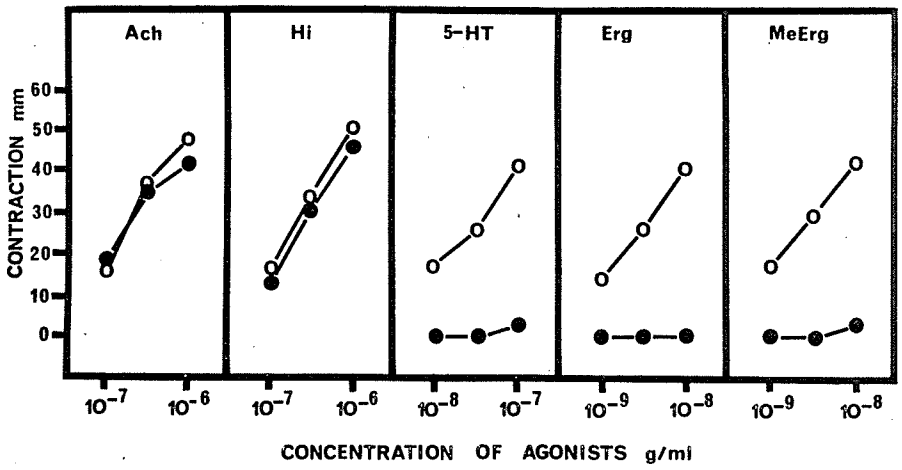

Fig. 18. EFFECT OF BOL ON RESPONSES OF DOG URINARY BLADDER STRIP TO AGONISTS.

Log dose-response curves before BOL o — o, log dose-response curves with BOL (10^{-8} g/ml) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

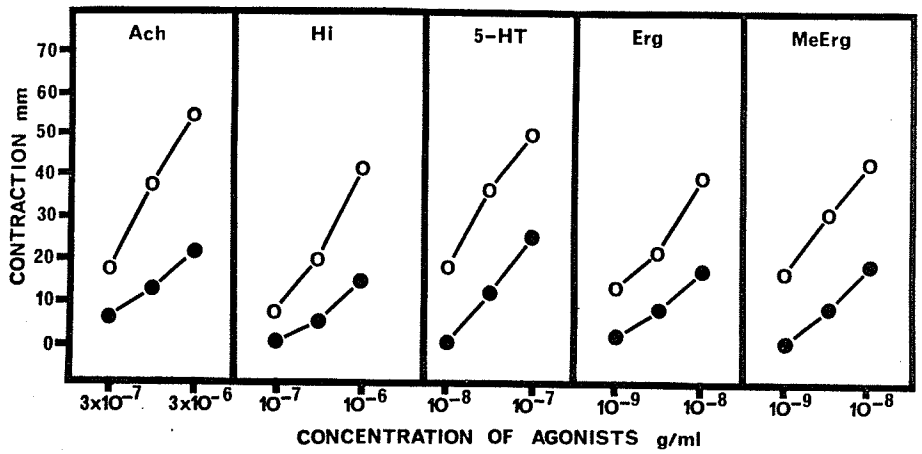

Fig. 19. EFFECT OF PHENOXYBENZAMINE ON RESPONSES OF DOG URINARY BLADDER STRIP.

Log dose-response curves before phenoxybenzamine O — O, log dose-response curves after phenoxybenzamine (10^{-7}g/ml) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

ergic receptors.

Almost complete abolition of responses to 5-HT, ergonovine, and methylergonovine by BOL suggested that the common receptor site involved in the contractile response of the strips to these drugs was of D type, and that there might be no M type receptors in dog urinary bladder strips. Gyermek (1962) who used a different technique of studying the effect of 5-HT on contractions of in vivo urinary bladder elicited by electrical stimulation of the pelvic nerve, postulated the presence of both M and D types of receptors in dog urinary bladder. To clarify this anomaly, the effects of morphine and atropine were tested in bladder strips. Neither morphine 10^{-6} g/ml (Fig. 20) nor atropine 10^{-8} g/ml antagonized the actions of 5-HT or ergot alkaloids indicating the presence of only the D type receptors.

Diphenhydramine (10^{-7} g/ml), which almost completely abolished the responses to histamine, had no effect on the responses to the other agonists showing that the stimulatory effect of ergot alkaloids is not mediated through release of histamine or by action on histamine receptors.

Determination of pA_2 with BOL. Antagonism studies with BOL suggested that 5-HT, ergonovine, and methylergonovine acted on the same receptors in dog urinary bladder strips. Since drugs which act on the same receptors give rise to the same pA_x when tested with a competitive antagonist (Schild, 1957), pA_2 values of BOL against the agonists were therefore determined in this preparation.

Before actual determination of pA_2 values could be done, it was necessary to establish the type of antagonism between BOL and 5-HT.

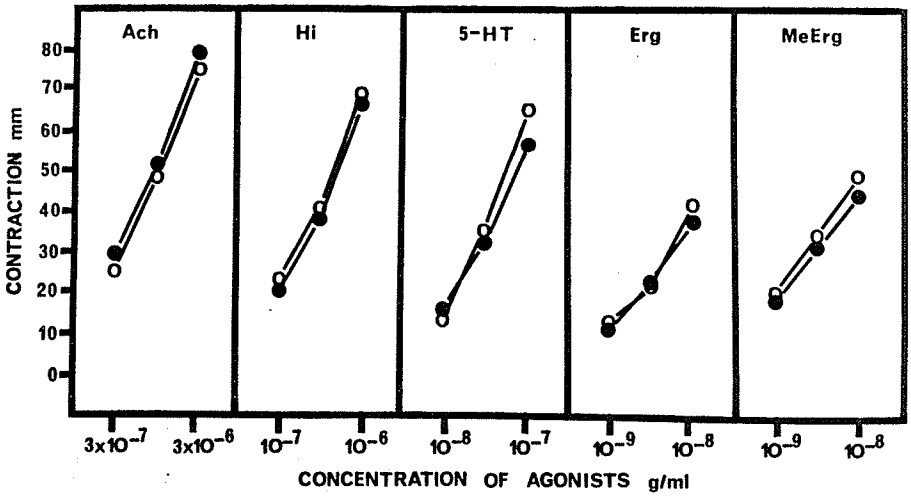

Fig. 20. EFFECT OF MORPHINE ON RESPONSES OF DOG URINARY BLADDER STRIP TO AGONISTS.

Log dose-response curves before morphine O — O, log dose-response curves with morphine ($5x10^{-7}$ g/ml) ● — ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

Therefore the effect of BOL in different concentrations on full dose-response curves of 5-HT was studied in four experiments. In concentrations from 10^{-10} to 10^{-8} g/ml, antagonism by BOL is surmountable; the dose-response curves are shifted to the right and are quite parallel to the control curve. On washout of the BOL the control curves were reobtained and these data show the antagonism by BOL against 5-HT is competitive.

The results of pA_2 determinations are summarized in Table III. The figures given are mean values, the total number of individual determinations and their standard deviation being indicated in the table. pA_2 values of BOL against 5-HT, ergonovine, and methylergonovine approximate each other and on this basis these three agonists can be grouped together. BOL sharply discriminates this group from other agonists, acetylcholine and histamine.

Anti-5-Hydroxytryptamine Activity of Ergot Alkaloids. The effect of different concentrations (10^{-11} to 3×10^{-10} g/ml) of ergot alkaloids on dose-response curves of 5-HT was studied in five experiments. The results of a representative experiment are shown in Fig. 21. All the ergot alkaloids tested are potent antagonists of 5-HT in dog urinary bladder strips. A low concentration such as 10^{-11} g/ml, which has no effect on the spontaneous activity of the preparation, is effective in antagonizing the contractile responses to all the tested doses of 5-HT. The antagonism against 5-HT was specific since responses to acetylcholine or histamine were not depressed by 3×10^{-10} g/ml of ergot alkaloids. These results are in agreement with the observations of Fingl and Gaddum (1953) and Cerletti and Doepfner (1958) regarding the antagonistic effects of ergot compounds against 5-HT in isolated rat uterus.

TABLE III

ANTAGONISM BY 2-BROMOLYSERGIC ACID DIETHYLAMIDE IN DOG URINARY BLADDER

AGONISTS	pA_2 VALUES (MEAN ± S.E.)	NUMBER OF DETERMINATIONS
5-Hydroxytryptamine	8.06 ± 0.054	11
Ergonovine	8.16 ± 0.087	8
Methylergonovine	8.25 ± 0.129	7
Histamine	4.45 ± 0.129	7
Acetylcholine	3.65 ± 0.185	6

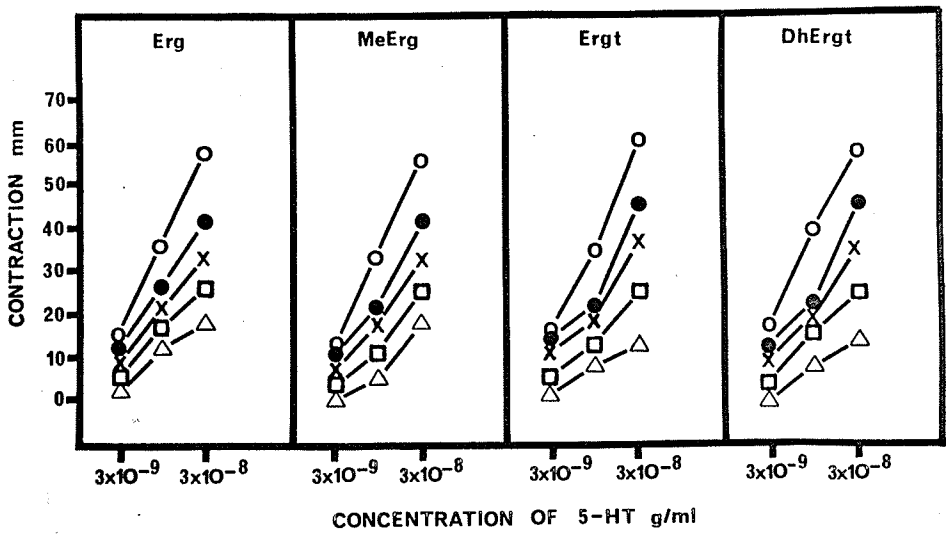

Fig. 21. ANTAGONISM OF 5-HT BY ERGONOVINE, METHYLERGONOVINE, ERGOTAMINE, AND DIHYDROERGOTAMINE.

Log dose-response curves of 5-HT without antagonist O – O, log dose-response curves of 5-HT with 1×10^{-11} g/ml of the antagonist ● – ●, log dose-response curves of 5-HT with 3×10^{-11} g/ml of the antagonist x – x, log dose-response curves of 5-HT with 1×10^{-10} g/ml of the antagonist □ – □, log dose-response curves of 5-HT with 3×10^{-10} g/ml of the antagonist Δ – Δ.

5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg; Ergotamine, Ergt; Dihydroergotamine, DhErgt.

Relationship between the Degree of 5-HT Antagonism and the Exposure Time to the Inhibitory Substances. Dose-ratios for 5-HT were determined at intervals of twenty minutes during the continued exposure of the bladder strips to a constant concentration (10^{-10} g/ml) of one of the three antagonists BOL, ergonovine, and methylergonovine. The relationship between the degree of 5-HT antagonism and the exposure time to the inhibitory agents was studied by plotting the dose-ratios against time. Fig. 22 shows the curves for BOL, ergonovine and methylergonovine. Each curve represents the mean of five experiments. It is evident that the behaviour of BOL, which is structurally related very closely to ergonovine and methylergonovine, is quite different. The dose-ratio increases continuously in case of BOL while it remains fairly constant with ergonovine and methylergonovine.

Effect of Reserpine Pretreatment. Doses of ergonovine and methylergonovine which caused contractions in urinary bladder strips from normal animals produced contractions of the strips from reserpine pretreated animals. These observations exclude the somewhat unlikely possibility that these ergot alkaloids may act indirectly through release of endogenous 5-HT.

Fig. 23 shows the mean results of five experiments in which contractile responses to various agonists were recorded in preparations taken from reserpine pretreated as well as untreated animals. Responses to various graded doses of all agonists tested were potentiated in preparations obtained from reserpine pretreated animals. These results are in agreement with similar observations in guinea-pig ileum.

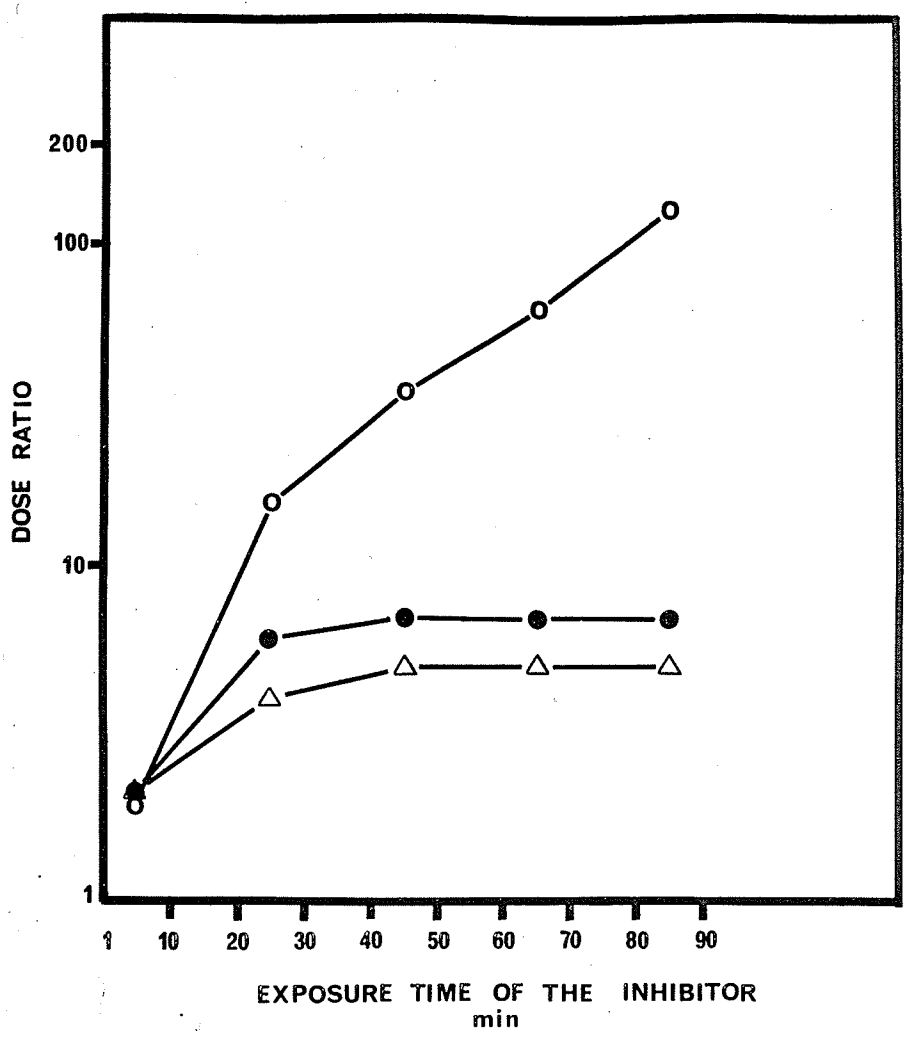

Fig. 22. THE INFLUENCE OF DURATION OF EXPOSURE ON THE DEGREE OF INHIBITION BY ERGONOVINE, METHYL-ERGONOVINE AND BOL IN DOG BLADDER.

Each curve represents the mean of five experiments. In each experiment three bladder strips were taken from the same dog, one exposed to BOL ($1x10^{-10}$g/ml), O – O ; another to ergonovine ($1x10^{-10}$g/ml), ● – ● ; the third to methylergonovine (10^{-10}g/ml), Δ – Δ.

Each point represents a dose ratio referred to a control response to 5-hydroxytryptamine (10^{-8} g/ml), measured before addition of antagonist.

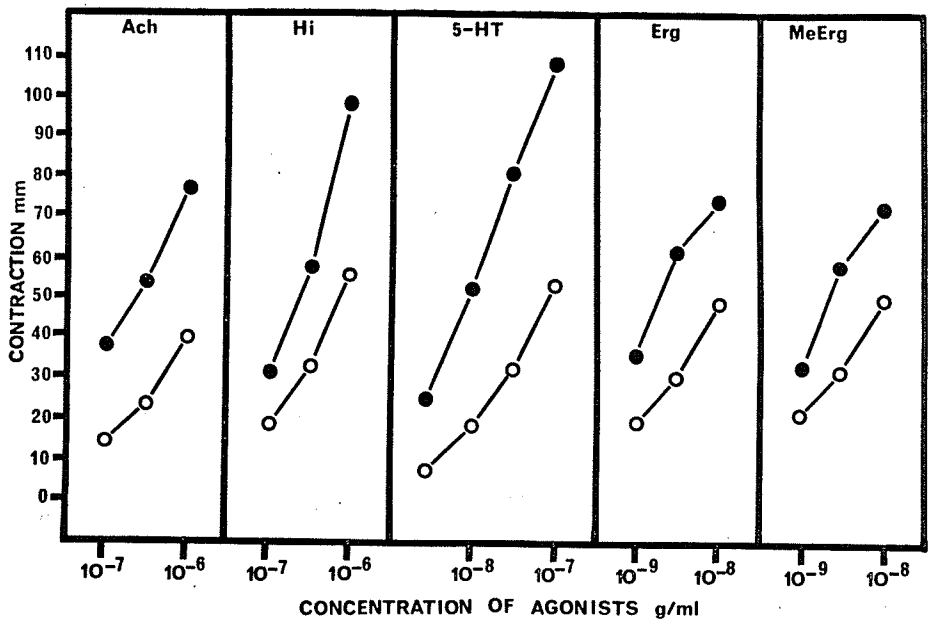

Fig. 23. EFFECT OF RESERPINE PRETREATMENT ON RESPONSES OF DOG URINARY BLADDER STRIP TO AGONISTS.

Log dose-response curves of untreated urinary bladder O – O , log dose-response curves of reserpine pretreated urinary bladder (1 mg/kg/24 hours) ● – ●.

Acetylcholine, Ach; Histamine, Hi; 5-Hydroxytryptamine, 5-HT; Ergonovine, Erg; Methylergonovine, MeErg.

SECTION IV

DISCUSSION

CHAPTER 7

DISCUSSION

A. GUINEA-PIG ILEUM

B. DOG URINARY BLADDER

The present investigation has been concerned with the determination of the type of receptors for a particular group of drugs, the ergot alkaloids. A brief description of drug-receptor interaction was given in the Introductory Section and the results will be discussed within this framework.

Two types of smooth muscle, guinea-pig ileum and dog urinary bladder, were used in the present study. In spite of a number of morphological and physiological differences between these two tissues, there is an important pharmacological similarity between them. Both tissues are inhibited by catecholamines and excited by the other biogenic agonists, acetylcholine, histamine, and 5-HT. On account of the similarity, the differentiation of receptors for ergot alkaloids in the two tissues presented a somewhat similar problem.

Many investigations on isolated and in vivo preparations have indicated that smooth muscle has specific receptors for acetylcholine, histamine, 5-HT, and catecholamines. In the present study the possibility of catecholamines receptors being the site of stimulatory action of ergot alkaloids was most unlikely, since both test preparations are inhibited by catecholamines. The mechanisms involved in pharmacological actions of ergot alkaloids might therefore be direct activation of receptors for acetylcholine, histamine or 5-HT or indirectly through the release of any of these agonists which then activated specific receptors. However, a possibility that ergot alkaloids were acting directly on some other type of receptors which are not yet fully characterized, had also to be considered.

Of the four ergot alkaloids investigated, only ergonovine and methylergonovine caused contractions of both the guinea-pig ileum and dog

urinary bladder. Results of the present study clearly demonstrate that ergonovine and methylergonovine act only on 5-HT receptors in both tissues to produce their stimulatory effect.

A. GUINEA-PIG ILEUM

The conclusion that 5-HT, ergonovine, and methylergonovine act on the same receptor sites in guinea-pig ileum is based on the striking evidence of cross-desensitization between 5-HT and ergot alkaloids. The ergot alkaloids failed to cause contraction of guinea-pig ileum which had become desensitized to 5-HT. Similarly 5-HT failed to cause contraction of guinea-pig ileum which had become desensitized to ergonovine or methylergonovine. Cross-desensitization between 5-HT and tryptamine was taken by Gaddum (1953) and Rocha e Silva *et al* (1953) as evidence for common site of action on specific receptors. Cross-desensitization between 5-HT and ergot alkaloids in the present study may similarly be considered as very strong evidence for ergonovine and methylergonovine acting on the same receptors as 5-HT. However, an unspecific depression of smooth muscle has been shown after large doses of an agonist (Cantoni and Eastman, 1946), and this possibility had to be considered. This possibility was excluded by the observation that desensitization by 5-HT or ergot alkaloids reduced the effects of acetylcholine or histamine only slightly but almost completely abolished responses to ergot alkaloids and 5-HT. The present results regarding specific desensitization of guinea-pig ileum after exposure to large concentrations of 5-HT are in complete agreement with those previously reported by Gaddum (1953), Rocha e Silva *et al* (1953), Vane (1960) and Brownlee and Johnson (1963). Exposure to 2×10^{-6} g/ml of 5-HT caused complete desensitization to 5-HT

but reduced responses to high doses of acetylcholine or histamine by only 10 % and the lower doses by 30 %. Almost complete abolition of responses to ergonovine or methylergonovine of the tissues desensitized by 5-HT and similar results with desensitization by ergot alkaloids is therefore strongly indicative of a common point of attack by 5-HT and ergot alkaloids.

The results of present antagonism studies also point to the same conclusion that 5-HT and ergot alkaloids act on the same receptors in guinea-pig ileum. These studies also exclude the involvement of specific receptors for acetylcholine or histamine.

Before the relative contribution of M and D receptors in the production of contractile responses of guinea-pig ileum to ergot alkaloids can be assessed, it seems desirable to discuss the current status of the original hypothesis of M and D receptors. Gaddum and Picarelli (1957) showed that guinea-pig ileum contained two kinds of receptors sensitive to 5-HT; one type, D receptors, could be blocked by Dibenzyline or lysergic acid diethylamide, and the second, M receptors, could be blocked by morphine, atropine or cocaine. It was suggested that D receptors were situated in the smooth muscle and M receptors in nervous tissue of the intestine. Recent reports, although agreeing with the hypothesis of two types of 5-HT receptor, disagree on their location. Day and Vane (1963) suggested that 5-HT receptors were mostly located in the nervous tissue. They had found that during anoxia, which inactivated the nervous tissue, the dose of 5-HT had to be increased about 500 fold to produce a response equal to the responses under control condition, while the sensitivity to acetylcholine or histamine was hardly modified. In view of their studies with antagonists, however, they concur-

red with Gaddum and Picarelli that there were two types of 5-HT receptors, but concluded that both were located in the nervous elements.

Harry (1963) and Brownlee and Johnson (1963) presented evidence to show that 5-HT mainly acts through specific receptors on intramural autonomic ganglia. They confirmed the high specificity of self desensitization by 5-HT. They found that competitive ganglion blocking agents did not antagonize the effects of 5-HT but the depolarizing agents as well as hyoscine did so. They therefore concluded that 5-HT activated specific receptors in the intramural parasympathetic ganglion cells. The negative evidence against D receptors shown by them was the lack of antagonism of 5-HT by lysergic acid derivatives. The last observation is, however, contrary to those of Gaddum and Picarelli (1957), Barlow and Khan (1959) and Kohli (1965).

It is apparent from the above discussion that the hypothesis of M and D receptors as presented by Gaddum and Picarelli (1957) is no longer uncontested. The results of antagonism studies will therefore be discussed in the light of these recent modifications of the original theory of M and D receptors.

Atropine, in a concentration of 10^{-8} g/ml, blocked the actions of acetylcholine and 5-HT but the actions of histamine and ergot alkaloids were not modified. From these experiments it may be inferred that ergot alkaloids do not act either on muscarinic acetylcholine receptor or indirectly through an action on some part of intramural nerve pathways involving the release of acetylcholine. The present finding that the action of histamine was not affected by this concentration of atropine conforms with the conclusions of others (Feldberg, 1951; Day and Vane, 1963; Brownlee and Johnson, 1963) that histamine acts directly on the

smooth muscle.

As regards antagonism of 5-HT by atropine, the present results agree with those of Rocha e Silva et al (1953) who showed that atropine blocked stimulation of the gut induced by 5-HT. Brownlee and Johnson (1963) who used hyoscine instead of atropine obtained similar results. Gaddum and Picarelli (1957) on the other hand obtained 50% residual response after atropine. These inconsistencies may be accounted for by the different experimental conditions and also by the observations of Cambridge and Holgate (1955) who found that there are two components of 5-HT action, one blocked by small concentrations of an antimuscarinic agent and the other blocked by high concentrations only. The concentration of 10^{-8} g/ml of atropine used in the present study falls into the lower range of concentrations of atropine used in the study by Cambridge and Holgate (1955) and yet the responses to 5-HT were markedly depressed.

Cocaine, in a concentration sufficient to block the action of 5-HT, had only a slight effect on the action of ergot alkaloids. When segments of guinea-pig ileum were exposed to 5×10^{-8} g/ml of cocaine, the dose of 5-HT had to be increased some 20 fold to produce a response equal to the response under control conditions. The corresponding dose-ratios for ergonovine and methylergonovine were only 1.5 and 1.4 respectively. Rocha e Silva et al (1953) found that cocaine did not affect the responses to histamine or acetylcholine, in concentrations that completely blocked the actions of 5-HT. Brownlee and Johnson (1963) showed that procaine, in a concentration sufficient to block the effects of nicotine, choline phenyl ether and DMPP, also blocked that of 5-HT. These

reports indicate the site of action of 5-HT is mainly on the nerve pathways, a conclusion also reached by Day and Vane (1963) from their experiments with morphine and with anoxia. Lower dose-ratios for ergonovine and methylergonovine as compared to those for 5-HT in the present studies may then be interpreted to mean that ergot alkaloids act on 5-HT receptors in the muscle.

The mean dose-ratio for 5-HT in the presence of 5×10^{-7} g/ml of morphine was found to be about 29 in the present studies. The corresponding dose-ratios for ergonovine and methylergonovine were 2.3 and 1.7 respectively. Actions of acetylcholine and histamine were hardly affected. As regards standard agonists, these results are in general agreement with those of Gaddum and Picarelli (1957), Kosterlitz and Robinson (1958), Barlow and Khan (1959) and Day and Vane (1963), although different concentrations of morphine were used by various workers. The exact significance of slight blockade of ergot alkaloids by morphine as compared to marked inhibition of 5-HT effect is not very clear at the present. The nature of morphine action is not well understood. Although most authors agree that morphine inhibits the release of acetylcholine from the nerve axon, the site and the mechanism of this action is still unknown (for references see Kosterlitz and Wallis, 1964). Moreover, morphine has been shown to cause unspecific block of the non-nicotinic receptors in the intramural ganglia (Trendelenburg, 1957). Lewis (1960) showed that morphine possessed more general depressant properties than were supposed. Day and Vane (1963) showed that morphine only partly antagonizes 5-HT receptors in nervous tissue. In view of such uncertainty about the nature and site of action of morphine, it is difficult to interpret these results in a precise manner.

BOL (5×10^{-6} g/ml) markedly antagonized 5-HT, ergonovine, and methylergonovine. Responses to acetylcholine were rather potentiated and those to histamine remained unaltered. These results regarding the effect of BOL on responses to 5-HT, agree with those of Gaddum and Picarelli (1957) and Barlow and Khan (1959) and suggest a common receptor site for 5-HT and ergot alkaloids.

Gaddum and Hameed (1954) showed that ergot alkaloid derivatives, in particular lysergic acid diethylamide, were specific 5-HT antagonists in rat uterus and rabbit ear but had little effect on guinea-pig ileum. Costa (1956) and Delay and Thullier (1956) obtained a potentiation of 5-HT with lysergic acid diethylamide on rat uterus. Other investigators (Cerletti and Doepfner, 1958) discovered a blocking action only. Brownlee and Johnson (1963) reported that lysergic acid diethylamide and its bromo-derivative did not produce any antagonism of 5-HT in guinea-pig ileum in concentrations less than 10^{-6} g/ml. Higher concentrations of BOL induced an intense spasm and increase of tone. In the present study, 5×10^{-6} g/ml of BOL caused only a slight increase in tone, which, however, did not interfere with the measurement of responses to various agonists. The present results thus differ from those of Brownlee and Johnson (1963) in this respect and agree with those of Kohli (1965) who showed that BOL did not produce intense spasm so as to render the preparation unworkable and further BOL did antagonize 5-HT.

Treatment of guinea-pig ileum with phenoxybenzamine (10^{-7} g/ml) more or less completely blocks the D receptors (Gaddum and Picarelli, 1957). When guinea-pig ileum was exposed to this concentration of phenoxybenzamine for five minutes, the responses to all the agonists tested were reduced; the dose-ratios were 2.6 for 5-HT, 2.6 for acetylcholine,

2.8 for histamine and more than 30 for ergonovine and methylergonovine. As far as acetylcholine, histamine and 5-HT are concerned, these results are in general agreement with those of Day and Vane (1963) and Brownlee and Johnson (1963). Marked antagonism by phenoxybenzamine against ergot alkaloids as compared to its effect against other agonists and blockade by BOL of responses to 5-HT as well as ergot alkaloids without any effect on acetylcholine or histamine responses, strongly suggest that ergonovine and methylergonovine act on D type receptors for 5-HT in guinea-pig ileum.

The observation that phenoxybenzamine (10^{-7} g/ml) blocked responses to ergonovine and methylergonovine to a greater extent than those to 5-HT suggested that there might be an adrenergic component in the stimulatory action of these alkaloids in addition to their activation of D type 5-HT receptors. Innes (1962a) presented evidence that, in smooth muscle where adrenaline stimulates, both ergotamine and ergonovine act by combining with adrenergic α-receptors. The possibility of ergonovine and methylergonovine activating α-receptors had to be excluded because at time of doing the experimental work, there was no information about receptors subserving relaxation in guinea-pig ileum. It has since been shown that both α and β adrenergic receptors in guinea-pig ileum subserve inhibition (Wilson, 1964; Kosterlitz and Watt, 1964). In the present study, the adrenergic blocking agent, phentolamine (10^{-8} to 10^{-6} g/ml) had no specific antagonistic effect against the stimulatory action of ergonovine and methylergonovine. These experiments thus exclude the possibility of ergonovine and methylergonovine acting on adrenergic α- receptors to produce their contractile effect.

The competitive ganglion blocking agent, hexamethonium (5×10^{-6} g/ml), had no effect on responses of guinea-pig ileum to 5-HT, acetylcholine or histamine. These results agree with those of Feldberg (1951), Rocha e Silva et al., (1953), Robertson (1953), Gaddum and Hameed (1954), Kosterlitz and Robinson (1958), Day and Vane (1963) and Brownlee and Johnson (1963). These experiments show it is unlikely that 5-HT has a preganglionic action but do not exclude the possibility that 5-HT may act through receptors in the ganglia which are not blocked by hexamethonium, as shown in the inferior mesenteric ganglion (Gyermek and Bindler, 1962) or at some part of the axon peripheral to the ganglion. Hexamethonium had no effect on responses of guinea-pig ileum to ergot alkaloids. A conclusion which may be drawn from these observations is that, if there is any nervous component in the action of ergot alkaloids, it is as resistant to hexamethonium as is 5-HT, and it is unlikely that 5-HT or the ergot alkaloids act on nicotinic ganglionic sites.

The ganglion-blocking agents that act by depolarizing the nerve cell would be expected to block the actions of any drug upon that cell regardless of the position of its receptor. Thus if the 5-HT receptors are situated on ganglion cells at some point away from the nicotine receptor, a depolarizing ganglion-blocking agent should prevent 5-HT from acting. DMPP in high concentrations is believed to block ganglia by depolarizing the ganglion cell (Ling, 1959). The observation that 5-HT contractions were markedly inhibited by the depolarizing action of DMPP seems acceptable evidence for siting the 5-HT receptor on the ganglion cell. Brownlee and Johnson (1963) made similar observations. Responses to ergot alkaloids were slightly depressed by DMPP (5×10^{-6} g/ml). This effect

was not specific as responses to acetylcholine and histamine were also slightly depressed. This is in accord with the observations derived from the present experiments with atropine and morphine that the major action of ergot alkaloids is likely to be directly on D type 5-HT receptors on the muscle.

Day and Vane (1963) presented evidence that 5-HT contracts guinea-pig ileum mainly through receptors in nervous tissue. Smooth muscle receptors were found not to contribute much towards contractile response to 5-HT unless the neuronal mechanisms were inactivated. Phenoxybenzamine which is supposed to block smooth muscle receptors, also antagonized some of the effects of 5-HT on nerves. D type receptors were thus present not only in the muscle cells but also in the nervous system. It was therefore suggested that the terms M and D receptors should not be quantitatively equated with nervous and smooth muscle receptors but should be restricted to their susceptibility to different antagonists.

Present studies were strongly indicative that the stimulatory response of guinea-pig ileum to ergonovine and methylergonovine was through the activation of D type receptors. To what extent D type receptors involved in this response were located in neuronal tissue was investigated by comparing responses to various agonists before and after storage of ileum at 4°C for 24-72 hours.

The responses to acetylcholine and histamine were not modified to a great extent by cold storage of the tissues. On the other hand, responses to 5-HT were markedly depressed. These results agree with the conclusions arrived at by various authors (Emmelin and Feldberg, 1947; Feldberg, 1951; Ambache and Lessin, 1955; Innes et al., 1957; Kosterlitz

and Robinson, 1958; Day and Vane, 1963) that acetylcholine and histamine act mainly through the direct excitation of smooth muscle whereas 5-HT acts mainly through receptors in nervous tissue.

In most experiments responses to the ergot alkaloids after cold storage of guinea-pig ileum were depressed but not so fully as were those to 5-HT. It may be inferred from these results that at least some D type receptors which are activated to produce contractile responses, are located in neuronal tissue. This would be in agreement with the conclusions arrived at by various authors (Innes et al., 1957; Day and Vane, 1963; Johnson, 1963) that cooling the guinea-pig ileum affects the responses of drugs acting on the intramural plexuses. The possibility that decrease in responses to ergot alkaloids may be actually due to changes in the sensitivity or contractility of the muscle fibres themselves, may be excluded by the observations that responses to acetylcholine and histamine under these conditions were not depressed to any great extent.

The effects of tyramine have been attributed by Burn and Rand (1958) to an indirect action, namely, by release of stored noradrenaline which then activates the smooth muscle. This view is based on evidence that tyramine has little action on smooth muscle depleted of noradrenaline. Innes (1962) presented evidence for 5-HT acting on adrenaline receptors in cat spleen strips by two mechanisms; viz., a major action due to release of stored noradrenaline and a minor direct action on adrenaline receptors. Since reserpine depletes guinea-pig ileum of its 5-HT content (Bulbring and Crema, 1959), a similar indirect action of ergot alkaloids by release of 5-HT was possible. This possibility was tested by comparing responses in preparations from reserpine pretreated and un-

treated animals. The responses to ergonovine and methylergonovine were not depressed in reserpine pretreated preparations; rather there was potentiation. It is concluded that the contractile response to the ergot alkaloids cannot be attributed to the release of endogenous 5-HT.

Responses to acetylcholine, histamine and 5-HT were also potentiated in such preparations. An interesting observation regarding the dose-response curves was made. Whereas a shift to the left of the dose response curve was plotted from responses of preparations from reserpine pretreated animals in case of acetylcholine and histamine, there was, in addition to a similar shift, an increase in the maximum responses with 5-HT, ergonovine and methylergonovine. No explanation is offered for this observation.

Some drugs, instead of being either pure agonists or pure antagonists may possess both agonistic and antagonistic activities. Such drugs have been called 'partial agonists' (Stephenson, 1956) and have been shown to possess intermediate intrinsic activity (Ariens, 1954; Ariens and Simonis, 1954; Stephenson, 1956; Ariens, van Rossum and Simonis, 1957; Ariens, 1964). van Rossum (1960) reported that pilocarpine is a partial agonist of acetylcholine. Innes (1962) presented evidence that ergotamine is an adrenaline antagonist with intrinsic activity, that is, one which acts as a partial agonist.

Both ergonovine and methylergonovine have been shown to be potent antagonists of 5-HT in rat uterus (Cerletti and Doepfner, 1958). The intrinsic activity of these compounds in guinea-pig ileum is evident from the contractile response of this tissue to them as shown in the present study. These observations led to the supposition that ergonovine

and methylergonovine might be partial agonists to 5-HT in guinea-pig ileum.

As partial agonists, the maximal height of dose-response curves of ergonovine and methylergonovine on guinea-pig ileum should necessarily be a fraction of that of 5-HT. This, as a matter of fact, appeared to be true, as may be seen from Fig. 2.

The dualistic nature of action of partial agonists implies that ergonovine and methylergonovine would act synergistically with low doses of 5-HT but as competitive antagonistics with high doses of 5-HT. When dose-response curves for ergonovine or methylergonovine in presence of various doses of 5-HT were plotted, this was found to be true. With low concentrations, of 5-HT, ergonovine acted as a synergist. When high concentrations of 5-HT were combined with graded doses of ergonovine, there was increasing antagonism at the higher dose-levels of ergonovine. The highest dose of ergonovine, independent of the concentration of 5-HT combined with it, always resulted finally in the same response, suggesting that ergonovine given in adequate concentration finally occupied all 5-HT receptors. The same was true of methylergonovine. These experiments thus further presented evidence for ergonovine, methylergonovine and 5-HT acting on the same receptors in guinea-pig ileum.

It is evident from the aforementioned discussion that contractile response of guinea-pig ileum to ergonovine and methylergonovine is due to activation of D type receptors. Ergotamine and dihydroergotamine did not manifest stimulatory effect upon guinea-pig ileum but exhibited antagonistic effect against various agonists. Both ergotamine and dihydroergotamine depressed 5-HT responses. This observation is in general agree-

ment with Gaddum and Picarelli (1957) who found dihydroergotamine antagonistic towards 5-HT responses in guinea-pig ileum. They presented evidence that dihydroergotamine blocks D but not M receptors.

Terminal segments of guinea-pig ileum respond to noradrenaline by contraction, an α adrenergic activity. This activity was totally abolished by 10^{-7} g/ml of ergotamine or dihydroergotamine in the present study. Nonterminal segments of guinea-pig ileum respond to catecholamines by inhibition. Kosterlitz and Watt (1964) and Wilson (1964) have presented evidence for the presence of both α and β inhibitory adrenergic receptors in nonterminal segments of guinea-pig ileum. Effect of both types of adrenergic blocking agents on inhibitory responses of noradrenaline and isoprenaline was investigated. Noradrenaline inhibits the gut by acting mainly on α receptors, while isoprenaline does so by acting on β receptors only. The percentage decrease in the height of contraction produced by coaxial stimulation of the gut was taken as the normal response of noradrenaline or isoprenaline. Responses before and after addition of blocking agents were compared. α-adrenergic blocking agents, ergotamine or dihydroergotamine had no effect on isoprenaline responses but noradrenaline responses were partially blocked. The adrenergic blocking agent, pronethalol completely blocked the isoprenaline responses but only partially blocked noradrenaline responses. Presence of both α and β adrenergic blocking agents was necessary to abolish noradrenaline responses completely.

These results confirm the reports of Kosterlitz and Watt (1964) and Wilson (1964) regarding the presence of both α and β inhibitory adrenergic receptors in guinea-pig ileum, but are not in full agreement

with Rothlin et al (1954), who reported that ergotamine and dihydroergotamine were specific antagonists of the inhibitory response of adrenaline and noradrenaline in rabbit small intestine.

B. DOG URINARY BLADDER

Erspamer (1953) first reported that 5-HT stimulates isolated urinary bladder of dog and that 5-HT responses were almost abolished by Dibenamine in a concentration which had no effect on responses to acetylcholine. Gyermek (1961), who studied the pelvic nerve-bladder preparation in situ of the dog, reported that 5-HT was a more powerful stimulant of dog urinary bladder than any other biogenic amine. He showed that atropine and hexamethonium had no effect on the stimulant action of 5-HT and concluded that 5-HT does not act directly or indirectly, through the liberation of acetylcholine, on the cholinergic receptor sites of the bladder. In a later paper (Gyermek, 1962) he reported the effects of various antagonists and postulated the existence of both M and D types of 5-HT receptors in dog urinary bladder, though he did not comment on their possible locations.

Results of the present study, however, indicate that the situation in dog urinary bladder, as regards 5-HT, is quite different from guinea-pig ileum. Morphine (10^{-7} g/ml) had no effect on 5-HT responses in bladder strips. Atropine in a concentration which completely abolished responses to acetylcholine had no effect on 5-HT responses. This agrees with earlier reports of atropine-resistant stimulatory effect of 5-HT in dog bladder (Gyermek, 1961; 1962).

In contrast with guinea-pig ileum, the atropine-resistant

nature of the 5-HT effect in dog bladder is a property which is shared with many tissues, e.g., rat uterus (Gaddum and Hameed, 1954), rat stomach (Vane, 1957), cat spleen (Innes, 1962) and guinea-pig trachea (Constantine and Knott, 1964). Similarity of the 5-HT effect in these tissues extends beyond resistance to atropine. Morphine has no antagonistic effect in rat stomach and cat spleen. BOL, a potent and specific antagonist of 5-HT in rat uterus (Cerletti and Doepfner, 1958) blocks D receptors for 5-HT but not the M receptors (Gyermek, 1961). BOL when tested for 5-HT antagonism on dog bladder, was found to be a potent antagonist. Phenoxybenzamine also markedly inhibited 5-HT responses.

The failure of morphine and atropine to antagonize the effect of 5-HT indicates that there may be no M type receptors in dog urinary bladder. This is in contradiction with the suggestion of Gyermek (1962) about the presence of both M and D receptors in dog urinary bladder. As a widely different technique was used by Gyermek (1962) as compared to the present study, it is impossible to pinpoint the cause of discrepancy in the two studies.

Responses of dog bladder to ergonovine and methylergonovine were affected by antagonists in the same manner as were 5-HT responses. BOL and phenoxybenzamine markedly inhibited responses to ergonovine and methylergonovine but atropine and morphine had no antagonistic effect. These findings suggest that ergonovine and methylergonovine act in dog bladder on the same receptor site as 5-HT and the common receptor involved is the D type.

Since drugs which act on the same receptors give rise to the same pA_x when tested with an antagonist (Schild, 1957), the similarity

of pA_2 values of BOL against ergonovine, methylergonovine and 5-HT determined in the present study confirm that they act on the same receptors.

As is evident from the present series of experiments BOL pA_2 value 8.06 proved to be a potent antagonist of 5-HT in dog bladder, approximately as potent as lysergic acid diethylamide in rat uterus -- pA_2 value of lysergic acid diethylamide against 5-HT in isolated rat uterus was 8.7 (Gaddum and Hameed, 1954).

Experiments with the adrenergic blocking agent, phentolamine, excluded the possibility of ergonovine and methylergonovine acting on adrenergic α-receptors to produce their contractile effect. The only effect observed was slight depression of all responses to all agonists, an unspecific effect.

The antihistaminic agent, diphenhydramine had no effect on responses to ergot alkaloids. This excluded the possibility of ergonovine and methylergonovine acting directly on histamine receptors or indirectly through release of endogenous histamine.

It follows, from what has been said above, that the stimulatory effect produced by ergonovine and methylergonovine is due to activation of 5-HT receptors. This may be brought about by ergot alkaloids acting directly on the receptors or indirectly through release of endogenous 5-HT which would then activate specific receptors. To clarify this point, responses to ergonovine and methylergonovine in preparations depleted of 5-HT by reserpine pretreatment were compared to responses in untreated preparations. This possibility of indirect action of ergot alkaloids through release of endogenous 5-HT was excluded because responses to ergot alkaloids were not inhibited; rather these were poten-

tiated. These results are in agreement with similar observations in guinea-pig ileum.

Antagonistic activity of ergot alkaloids, ergotamine, dihydroergotamine, ergonovine, and methylergonovine against 5-HT in dog urinary bladder was also studied. All the ergot alkaloids tested were found to be potent antagonists of 5-HT in this preparation. A low concentration such as 10^{-11} g/ml, which had no effect on the spontaneous activity of the preparation, reduced the contractile responses to all the tested doses of 5-HT. The antagonism against 5-HT was specific since responses to acetylcholine or histamine were not depressed. These results are in general agreement with the observations of Fingl and Gaddum (1953) and Cerletti and Doepfner (1958) regarding the antagonistic effects of ergot compounds against 5-HT in isolated rat uterus.

The degree of blockade of 5-HT responses by BOL changed appreciably with the exposure time. However, with ergonovine and methylergonovine, which are structurally very closely related to BOL, maximum antagonism was achieved soon and then it remained constant. Similar observations were made by Gaddum et al (1955) and Cerletti and Doepfner (1958) in rat uterus.

Several of the standard techniques of drug receptor analysis have been applied to this study on ergot alkaloids. The results derived from these different approaches have consistently led to the same conclusion, namely that ergonovine and methylergonovine produce their contractile response in guinea-pig ileum and dog urinary bladder by acting directly on 5-HT receptors, and that the action is predominantly on the D type of 5-HT receptors.

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