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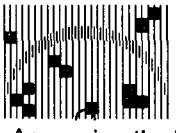

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City University of New York, 1987

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A PHARMACOLOGICAL CHARACTERIZATION OF THE 5-HYDROXYTRYPTAMINE$_2$ (5-HT$_2$) RECEPTOR IN THE ISOLATED RABBIT AORTA WITH TRYPTAMINE ANALOGS, AND COMPETITIVE AND NONSURMOUNTABLE ANTAGONISTS: ANALYSES WITH STEADY-STATE AND KINETIC METHODS

by

BRIAN M. CLANCY

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the City University of New York.

1987

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ABSTRACT

A PHARMACOLOGICAL CHARACTERIZATION OF THE 5-HYDROXYTRYPTMINE$_2$ (5-HT$_2$) RECEPTOR IN THE ISOLATED RABBIT AORTA WITH TRYPTAMINE ANALOGS, AND COMPETITIVE AND NONSURMOUNTABLE ANTAGONISTS: ANALYSES WITH STEADY-STATE AND KINETIC METHODS

by

BRIAN M. CLANCY

Adviser: Saul Maayani, Ph.D.

The 5-HT$_2$ receptor in the isolated rabbit aorta was characterized with eighteen tryptamine analogs and four competitive and two nonsurmountable antagonists of the response to 5-HT. Drug-receptor interactions were investigated with steady-state methods and a novel kinetics method. All tryptamine analogs were agonists and their dissociation constants and relative intrinsic efficacies were determined. Structure-activity observations revealed two trends. First, mono- and dimethylation of the side-chain nitrogen selectively decreased drug efficacy. Second, minor modifications of the indole ring resulted in parallel increases or decreases in drug affinity and efficacy. These results provide some information about structural requirements necessary for the development of competitive antagonists

from simple tryptamine derivatives which would be useful for the classification of 5-HT_2 receptors in other systems. The kinetics of agonist and antagonist interactions were studied by following antagonist-induced decreases in the steady-state response to an agonist. A model was fitted to the data and it yielded estimates of the association and dissociation rate constants of the agonist and the antagonist. Calculated dissociation constants (k_{-x}/k_x) agreed with those determined with steady-state methods. The kinetic rate constants of the high affinity antagonists were similar to those previously reported in high affinity binding studies. The results suggest that the association rate constant is the primary determinant of drug affinity and that the kinetic rate constants reflect molecular interactions of these drugs with the receptor. Lysergic acid diethylamide (LSD) and 2-iodo-LSD (IOL) were nonsurmountable antagonists of the response to 5-HT. Results of steady-state and kinetic experiments indicated that LSD and IOL bound to the 5-HT-recognition site on 5-HT_2 receptors. It is proposed that these antagonist-receptor complexes interact with the transducer system in an undefined manner, possibly resulting in the formation of a slowly reversible ternary complex.

Acknowledgements

This dissertation is dedicated to my wife Roxanne and to our daughter Emilia. Their almost infinite patience with me and our circum$tance$ during my years as a student helped make this work possibe and for that I am thankful.
Others who deserve my thanks and appreciation are:
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The following publications, abstracts and manuscripts were derived from parts of this dissertation:

1. Clancy, B.M. and Maayani, S.: 5-hydroxytryptamine receptor in isolated rabbit aorta: characterization with tryptamine analogs. J. Pharmacol. Exp. Ther. 233: 761-769, 1985.

2. Clancy, B.M., Osman, R. and Maayani, S.: Kinetics of competitive drug action at 5-hydroxytryptamine$_2$ receptors in isolated rabbit aorta. J. Pharmacol. Exp. Ther. IN PRESS.

3. Clancy, B.M and Maayani, S.: Nonsurmountable antagonism of the response to 5-hydroxytryptamine by d-lysergic acid diethylamide (LSD) and 2-iodo-LSD in isolated rabbit aorta: steady-state and kinetic analyses. SUBMITTED TO J. Pharmacol. Exp. Ther.

4. Clancy, B.M, Kline, T.B. and Maayani, S.: 5-hydroxytryptamine receptors in the isolated rabbit aorta: Further characterization and structure-activity observations with tryptamine analogs. SUBMITTED TO J. Pharmacol. Exp. Ther.

5. Clancy, B, Silva, W. and Maayani, S.: 5-HT receptors in isolated rabbit aorta: characterization by a series of 5-HT agonists. Fed. Proc. 42:1150, 1983.

6. Clancy, B., Osman, R. and Maayani, S.: Determination of agonist (Ag) or competitive antagonist (An) dissociation constants from competition kinetics at the 5-hydroxytryptamine$_2$ (5-HT$_2$) receptor in isolated rabbit aorta. Fed. Proc. 45: 435, 1986.

7. Maayani, S., Clancy B., Hyslop, D. and Yocca F.: Action of tryptamine and ergoline derivatives on functional 5-HT receptors in brain and periphery. Fed. Proc. 46: 966, 1987.

INTRODUCTION

Presently, there are at least three general classes of 5-HT receptors and they are designated as 5-HT_1-like, 5-HT_2 and 5-HT_3 (see reviews by Bradley et al., 1986; Green and Maayani, 1987). Among these classes of receptors, 5-HT_2 receptors are considered the most easily classified since there are a number of antagonists, e.g., spiperone, methysergide and ketanserin, with high affinities for this receptor. However, there appear to exist discrepancies in the pharmacological characteristics of 5-HT_2 receptors in different tissues and species. For example, some drugs are surmountable antagonists of the response to 5-HT in one tissue but are nonsurmountable antagonists in another; also the affinity of an antagonist for this receptor may differ significantly in different tissues (Leff and Martin, 1986). These discrepancies may or may not indicate receptor heterogeneity within this class but they do warrant a more complete pharmacological characterization of this receptor subtype. With this approach new pharmacological characteristics may be determined, with new drugs, which may in turn lead to revisons in existing classification schemes or in our understanding of drug-receptor interactions.

In the present investigation the interactions of agonists (tryptamine analogs) and competitive and nonsurmountable antagonists with 5-HT_2 receptors in

the isolated rabbit aorta are examined with steady-state and kinetic methods of analysis. The results of this investigation are discussed in terms of some of the structural requirements for recognition at 5-HT_2 receptors and activation of 5-HT_2 receptors by simple tryptamine analogs, the kinetics of agonist and antagonist interactions with this receptor and a possible explanation of the mechanism of action for two nonsurmountable antagonists of the response to 5-HT. What follows is a selective review of 5-HT_2 receptors with an emphasis on the importance of this study.

5-HT_2 binding sites.

The concept of multiple 5-HT binding sites was first proposed by Peroutka and Snyder (1979) who demonstrated the presence of two binding sites in homogenates of rat frontal cortex; these sites were designated as 5-HT_1 (d-LSD sensitive [^{3}H]-5-HT binding sites) and 5-HT_2 (d-LSD sensitive [^{3}H]-spiperone binding sites) binding sites. In general, both binding sites showed reciprocal pharmacological characteristics (Peroutka and Snyder, 1979). 5-HT_1 binding sites possessed higher affinities for 5-HT and its derivatives than for the 5-HT antagonists cyproheptadine, mianserin, cinanserin and spiperone. In contrast, 5-HT_2 binding sites possessed higher affinities for these antagonists

than for 5-HT and its derivatives. Early attempts to characterize 5-HT_2 binding sites were compromised by the lack of selective radioligands. For example, [^{3}H]-spiperone (Leysen et al., 1981) and [^{3}H]-mianserin (Peroutka and Snyder, 1981) were shown to have a higher affinities for dopamine-D_2 and histamine-H_1 binding sites, respectively, than for 5-HT_2 binding sites. The introduction of [^{3}H]-ketanserin, a potent 5-HT antagonist (Van Nueten et al., 1981) which bound selectively to 5-HT_2 binding sites (Leysen et al., 1981), made it possible to more rigorously characterize these binding sites. Leysen et al. (1982) determined the affinities of 65 drugs of various pharmacological classes for this site in membrane preparations from rat frontal cortex. From this study the affinities of several 5-HT antagonists have often been used as a classification standard for 5-HT_2 binding sites and receptors in such tissues as the rabbit aorta (Humphrey et al., 1982; Maayani et al., 1984), cat blood platelets (Leysen et al., 1983a), the rat caudal artery (Leysen et al., 1982) and the guinea pig trachea (Van Nueten et al., 1982).

5-HT_2 binding sites have been detected in the brains of several mammalian species such as rats (Leysen et al., 1982; Hoyer et al., 1985), humans (Schotte et al., 1983; Hoyer et al., 1986), guinea pigs (Leysen et al., 1982), pigs (Pazos et al., 1984), rabbits (Leysen et al.,

1983c) and dogs (Leysen et al., 1983c). In most of these species the greatest densities of sites were found in the frontal cortical areas, mesolimbic areas and striatum (Leysen et al., 1983c). Except for blood platelets from cats (Leysen et al., 1983a; 1983b) and humans (McBride et al., 1983; De Clerck et al., 1984a) the low densities of 5-HT_2 binding sites in peripheral tissues have prevented their detection with tritiated radioligands. Recently, [^{125}I]-2-iodo-LSD (IOL) was introduced as a selective radioligand for 5-HT_2 binding sites (Engel et al., 1984b; Kadan et al., 1984). The high specific radioactivity of [^{125}I]-IOL was supposed to make it possible to detect 5-HT_2 binding sites in peripheral tissues. The only attempts to examine the binding of [^{125}I]-IOL to a purported population of 5-HT_2 receptors in peripheral tissues were made with membrane preparations from the longitudinal muscle of the guinea pig ileum (Engel et al., 1984a; 1984b). The pharmacological characteristics of these [^{125}I]-IOL binding sites were not significantly correlated with those of [^{3}H]-ketanserin or [^{125}I]-IOL binding sites in rat brain cortex (Engel et al., 1984a). It appears that these [^{125}I]-IOL binding studies in the guinea pig ileum preparation were complicated by the presence of two 5-HT binding sites with high affinities for this radioligand. Indeed, it was demonstrated that the longitudinal muscle of the guinea pig ileum has two 5-HT

receptors; one that mediates contraction and is believed to be the 5-HT_2 receptor (Engel et al., 1984a; 1984b) and an undefined receptor that mediates relaxation (Feniuk et al., 1983; Kalkman et al., 1986). Both of these receptor-mediated responses were potently antagonized by IOL (Engel et al., 1984a; 1984b; Kalkman et al., 1986).

5-HT_2 receptors.

The response to 5-HT in various preparations is antagonized by drugs of several chemical classes, e.g., a quinazoline derivative (ketanserin), a butyrophenone derivative (spiperone) and an ergoline derivative (methysergide). Due to similarities in the potencies of these and other drugs which antagonize the response to 5-HT and which compete for 5-HT_2 binding sites, many functional preparations are believed to possess 5-HT_2 receptors. Table 1 presents an incomplete list of responses that are believed to be mediated by 5-HT_2 receptors.

Despite the diversity of species and tissues in table 1, biochemical studies indicate that some of these responses depend upon the same effector system. 5-HT stimulates the formation of inositol phosphates in the isolated rat aorta (Roth et al., 1984; Nakaki et al., 1985; Roth et al., 1986), myocytes from the rat aorta (Cory et al., 1986; Doyle et al., 1986) and human (De

Chaffoy de Courcelles et al., 1985) and rabbit blood platelets (Schachter et al., 1985). Studies with agonists and antagonists suggest that this biochemical response in rat aortic myocytes (Cory et al., 1986) and human blood platelets (De Chaffoy de Courcelles et al., 1985) is mediated by 5-HT_2 receptors. 5-HT also stimulates the formation of inositol phosphates in slices of rat cerebral cortex (Conn and Sanders-Bush, 1984; 1985; 1986; Kendall and Nahorski, 1985). Conn and Sanders-Bush (1984; 1985) reported that this response was mediated by 5-HT_2 receptors because it was antagonized by ketanserin, spiperone and other drugs. However, as shown in those studies (Conn and Sanders-Bush, 1984; 1985) and by Kendall and Nahorski (1985) the overall pattern of antagonist affinities was not entirely consistent with that observed at 5-HT_2 binding sites in membrane preparations from rat cortex. Despite this controversy, the work of Conn and Sanders-Bush (1984; 1985) and that of Kendall and Nahorski (1985) provides circumstantial evidence to suggest that 5-HT_2 receptors in brain tissue are coupled to the phosphatidylinositol second-messenger system.

In this system phosphatidylinositol 4,5-bisphosphate (PIP_2) is the central component of the receptor-transduction process. Briefly, PIP_2 is hydrolyzed by the agonist-dependent enzyme phospholipase C to form inositol 1,4,5-trisphosphate (IP_3) and

diacylglycerol (DG) which serve as intracellular second messengers. IP_3 stimulates the release of intracellular stores of calcium within the endoplasmic reticulum and DG activates protein kinase C. For additional information on agonist-stimulated phosphatidylinositol metabolism see the reviews by Downes (1986) and Abdel-Latif (1986).

Pharmacological discrepancies in the characteristics of 5-HT_2 receptors in different tissues.

As shown in table 1 the responses to 5-HT in a number of mammalian tissues are believed to be mediated by 5-HT_2 receptors. However, the classification of some of these receptors is tentative because antagonist dissociation constants (K_B) have not been determined in all preparations and correlated with dissociation constants (K_I) for the same drugs at 5-HT_2 binding sites. In many instances the antagonist concentration that inhibits fifty percent of the response (IC50) to a single concentration of 5-HT is determined and is correlated with the K_I values (note c in table 1). IC50 values have no molecular meaning since they depend upon the concentration of 5-HT used to produce the response. Thus, these correlations and possibly the conclusions drawn from them are questionable. In the strictest sense, the pharmacological classification of any

receptor requires that the K_B and K_I values show a significant correlation (note a in table 1). Such a rigorous requirement may not be practical for in vivo systems since it is difficult to quantify drug concentrations at the receptor level; for these systems an unambiguous classification of the 5-HT receptor(s) depends upon the development of agonists and antagonists with greater selectivity for 5-HT_2 receptors.

Perhaps some of the receptors in table 1 are not properly classified because some drugs are surmountable (simple competitive) antagonists of the response to 5-HT in some tissues but nonsurmountable antagonists in others. For example, spiperone, ketanserin and methysergide are competitive antagonists of the contractile response in the rabbit aorta (Humphrey et al., 1982; Maayani et al., 1984); in contrast, some or all of these drugs are nonsurmountable antagonists of the response (5-HT_2 receptor mediated) to 5-HT in the rat uterus (Ichida et al., 1983), human (Bevan and Heptinstall, 1985; 1986; Victorzon et al., 1986) and cat (De Clerck et al., 1984) blood platelets, the calf coronary artery (Kaumann and Frenken, 1985) and the calf trachea (Lemoine and Kaumann, 1986). Recently, Leff and Martin (1986) compared the actions of several 5-HT antagonists in three tissues reported to possess 5-HT_2 receptors. In their study, methysergide was a competitive antagonist in the rabbit aorta but was a nonsurmountable antagonist in the rat

caudal artery and the rat jugular vein. In addition, ketanserin was a competitive antagonist in the rabbit aorta and the rat jugular vein but was a nonsurmountable antagonist in the rat caudal artery. The reason(s) for the apparent tissue dependent behavior of these antagonists is not clear.

Recently, three hypotheses have been proposed to explain nonsurmountable antagonism of the response to 5-HT in tissues with 5-HT_2 receptors. First, 5-HT_2 receptors are allosterically modulated by nonsurmountable antagonists (Kaumann and Frenken, 1985; Lemoine and Kaumann, 1986). In this model the receptors exist in one of two interconvertible conformations, e.g., R and R'. 5-HT and competitive antagonists compete for a site on R and it is this conformation that produces most of the response to 5-HT. The nonsurmountable antagonist can only bind to a site (the allosteric site) distinct from the 5-HT-recognition site and this interaction causes the interconversion of receptors from the active (R) to the less active (R') conformation. Second, the nonsurmountable antagonist may slowly dissociate from the 5-HT_2 receptor (Bevan and Heptinstall, 1986). The pseudoirreversible binding of an antagonist to the receptor would effectively reduce the number of receptors available to the agonist and result in a lower maximal response. Third, the 5-HT_2 receptor couples to a signal transducer molecule (De Chaffoy de Courcelles et

al., 1986). In this model there are two binding sites on the receptor. One site (S_{out}) is exposed to the extracellular space and the other (S_{in}) is exposed to the intracellular environment. Agonists and antagonists compete for S_{out} while S_{in} serves as the binding site for an undefined transducer element. The formation of a nonsurmountable antagonist-receptor complex causes a conformational change (slowly reversible) in the receptor which prevents the binding of the transducer molecule to S_{in}. When the agonist replaces the nonsurmountable antagonist at S_{out} there is little effective coupling with the transducer at S_{in}. Thus, the maximal response is reduced.

These hypotheses were obtained from visual inspections of concentration-response curves (CRCs) obtained in the absence and in the presence of a nonsurmountable antagonist. However, this form of analysis does not yield reliable information about the nature of drug-receptor interactions. It should be noted that these hypotheses assume that only 5-HT_2 receptors mediate the response to 5-HT. This assumption seems justifiable only for the preparations already discussed.

Nonsurmountable antagonism can also occur when the antagonist binds selectively to one of several receptors which mediate the same response to the agonist. This type of situation has created some confusion in the classification of 5-HT receptors. In the isolated canine

basilar artery the contractile response to 5-HT was antagonized by many drugs, e.g., ketanserin, methysergide and spiperone, in a nonsurmountable manner (Peroutka et al., 1983; Muller-Schweinitzer and Engel, 1983). Peroutka et al. (1983) concluded that the response to 5-HT was mediated by 5-HT_1 receptors; in contrast, Muller-Schweinitzer and Engel (1983) concluded that this response was mediated by 5-HT_2 receptors. Although both groups observed nonsurmountable antagonism their conclusions differed because their experimental protocols were different. Muller-Schweinitzer and Engel (1983) obtained a highly significant correlation between antagonist IC50 values in the canine basilar artery and drug dissociation constants (K_I) at 5-HT_2 binding sites. Recall that this was how some preparations listed in table 1 were classified (note c). Recent analyses of the response to 5-HT in the canine basilar artery indicated that the response was mediated by both 5-HT_{1A} (Taylor et al., 1986; Peroutka et al., 1986) and 5-HT_2 receptors (Taylor et al., 1986; Peroutka et al., 1986; Frenken and Kaumann, 1986). In another example of this situation, Brazenor and Angus (1982) studied the contractile response to 5-HT in the isolated canine coronary artery and showed that drugs from a variety of chemical classes, e.g., ergot peptide alkaloids, lysergic acid derivatives and non-ergot compounds such as ketanserin and trazodone, were nonsurmountable

antagonists. They hypothesized that these drugs may antagonize the response by interfering in the translation of receptor stimulation into a biological response rather than by binding to the 5-HT recognition site on the receptor. However, Frenken and Kaumann (1985) showed that the antagonist effects of ketanserin were surmounted by high concentrations (mM) of 5-HT and when this occured the CRC of 5-HT was distinctly biphasic. Frenken and Kaumann (1985) concluded that the response to 5-HT in the canine basilar artery was mediated by at least two populations of 5-HT receptors, one population of which was the 5-HT_2 receptor.

Nonsurmountable antagonism can also occur when the antagonist has two independent and mutually antagonistic actions which occur in the same concentration range. For example, the antagonist may act as both a competitive and as a physiological antagonist. Leff and Morse (1987) showed that verapamil, a calcium channel antagonist, was a nonsurmountable antagonist of the contractile response to 5-HT in the isolated rabbit aorta. In the presence of verapamil the CRCs of 5-HT were shifted to the right and the maximal response to 5-HT was decreased. The nonsurmountable component of the antagonism produced by verapamil appears to be due to the drug's ability to block the entry of extracellular calcium into the smooth muscle cells (Fleckenstein, 1977). The competitive component of the antagonism produced by verapamil is due to the drug's

ability to bind to 5-HT_2 receptors (Auguet et al., 1986; Taylor and Defeudis, 1985).

The problem of nonsurmountable antagonism is not unique to the study of 5-HT_2 receptors; it is also observed in studies of other 5-HT receptors. For example, ICS 205-930, a drug with high affinity for peripheral neuronal 5-HT receptors, is a competitive antagonist of the response to 5-HT in the rabbit isolated vagus nerve (Richardson et al., 1985) but is a nonsurmountable antagonist on the rat isolated vagus nerve (Ireland and Tyers, 1987). In addition, the response to 5-HT is nonsurmountably antagonized by methysergide, mianserin and lysergic acid diethylamide (LSD) in the rat stomach fundus (Offermeier and Ariens, 1966; Frankhuijzen and Bonta, 1974; Clineschmidt et al, 1985). It is obvious from the preceding discussion that a single mechanism of action will not account for nonsurmountable antagonism of the response to 5-HT in all functional systems. Aside from the generality of this phenomenon studies of nonsurmountable antagonists are important for three reasons. First, nonsurmountable antagonists may provide additional insight into how an agonist generates the observed response. For example, the nonsurmountable antagonist effect may reveal that the agonist binds nonselectively to two populations of receptors. In this way it is possible that nonsurmountable antagonists may reveal new receptors for the agonist. Second, studies

with nonsurmountable antagonists may show receptor-independent actions of the antagonist that have physiological importance. Verapamil, a Ca^{++} channel/5-HT_2-receptor antagonist, exemplifies such a drug. Third, nonsurmountable antagonists may provide additional insight into the nature of drug-receptor or drug-receptor-tissue interactions. For example, is the nonsurmountable antagonist effect due to the kinetics of the antagonist-receptor interaction or does the antagonist prevent the agonist-receptor complex from coupling with a second messenger system? In addition to the problem of nonsurmountable antagonism, discrepancies in the values of dissocation constants of conventional antagonists suggest the need for an alternative approach to the pharmacological characterization and classification of 5-HT receptors.

<u>An alternative to conventional antagonists.</u>

Antagonists have long been regarded as the drugs of choice for the charactertization and subsequent classification of hormone receptors (Black et al., 1982). As discussed above the actions of some conventional 5-HT antagonists, e.g., ketanserin, spiperone and methysergide, differ between tissues reported to possess 5-HT_2 receptors. Many of these antagonists bear little or no chemical similarity to 5-HT (ketanserin and spiperone) or

are significantly larger (methysergide) than 5-HT. As Black (1987) has argued:

> "I expect that receptors will turn out to be similar to other reactive proteins, carriers, and so on, where only the reactive sites are highly conserved across species with respect to amino acid composition [Phillips et al., 1983]. Therefore, while we may be entitled to expect the functionality of hormones and their conjugate receptors to be highly conserved across species, no such expectation is reliable when exploring receptors with competitive antagonists ..."

because antagonists, by virtue of their molecular size, may bind to accessory sites around the agonist-recognition site on the receptor (Kenakin, 1984); sites which may not be highly conserved across species. For this reason agonists take on added importance and it is necessary to examine structure-activity relations for the natural agonist and its derivatives. With this alternative approach the classification of receptors can be assisted if such studies lead to the development of selective agonists and/or antagonists. A similar course of action led to the development of selective antagonists for the beta-adrenergic (Powell and Slater, 1958; Black and Stephenson, 1962), histamine-H_2 (Black et al., 1972) and serotonin-M (5-HT_3, Richardson et al., 1985) receptors. In this connection it is possible that an antagonist derived from the natural agonist would be less likely to show discrepant actions by virtue of its smaller molecular size. In support of this possibility it was shown that ketanserin and spiperone were nonsurmountable

antagonists of the 5-HT_2-receptor mediated formation of inositol phosphates in WRK1 (rat mammary tumor) cells; in contrast, the tryptamine analogs bufotenine and 5-methoxygramine were surmountable, competitive antagonists of the response to 5-HT (Cory et al., 1987). As mentioned previously, Leff and Martin (1986) examined the actions of four 5-HT_2 antagonists (trazodone, ketanserin, spiperone and methysergide) in the rabbit aorta and in the rat jugular vein. Methysergide was a competitive antagonist in the rabbit aorta but was a nonsurmountable antagonist in the rat jugular vein. The other three drugs were competitive antagonists in both preparations but their K_B values were statistically different in the two tissues, e.g., the K_B of trazodone was six times smaller in the rabbit aorta than in the rat jugular vein (Leff and Martin, 1986). As an alternative to these conventional antagonists, Leff et al. (1986) examined the actions of five tryptamine analogs (four agonists and one competitive antagonist) in the rabbit aorta and rat jugular vein. Each drug had the same dissociation constant and the agonists had the same relative intrinsic efficacies in both tissues.

Although binding studies are convenient for the study of structure-affinty relations of drugs there are two drawbacks when these methods are applied to agonists. First, when an agonist competes with a radiolabeled antagonist for a homogeneous population of binding sites

the slope of the concentration-inhibition curve of the agonist is often significantly less than the predicted value of one (Kent et al., 1980; Leysen et al., 1982). When this occurs the agonist dissociation constant (K_I) can not be accurately determined from the IC50 value and the Cheng-Prusoff (1973) equation. As with the beta-adrenergic (Kent et al., 1980), dopamine-D_2 (Sibley and Creese, 1982) and alpha$_1$-adrenergic (Goodhardt et al., 1982) binding sites, some studies have shown that 5-HT_2 binding sites are modulated by guanine nucleotides. In the presence of 0.1 mM of guanine nucleotides (GTP or its nonhydrolyzable analog Gpp(NH)p) the concentration-inhibition curves of agonists were shifted to the right of control curves and the slope values of the shifted curves were approximately one; the antagonist competition curves were not influenced (Battaglia et al., 1984; Titeler et al., 1984). It is believed that agonist sensitivity to the absence and presence of guanine nucleotides is due to the ability of the agonist-receptor complex to undergo an additional interaction with a component of the effector system which may be coupled to the activation or inhibition of adenylate cyclase or the activation of phospholipase C. Agonists stabilize or induce the formation of a complex between the receptor and a nucloetide regulatory (N) protein. In the absence of guanine nucleotides this complex persists and shows high affinity for agonists.

Guanine nucleotides destabilize this complex which results in the receptor having a lower affinity for the agonist (Lyon et al., 1987). Second, the apparent binding affinity of an agonist is influenced by other assay conditions, such as the concentations of various ions. 5-HT_2 binding sites in homogenates rat prefrontal cortex were modulated by variations in the concentrations of LiCl, NaCl and KCl (Battaglia et al., 1983a) and by changes in the pH (Battaglia et al., 1983b). In general, increases in the concentrations of the monovalent cations Na^+, Li^+, K^+ reduced the apparent affinity of 5-HT for the 5-HT_2 binding site; increases in the pH from 7.0 to 8.2 increased the apparent affinity of 5-HT and several other agonists for this binding site. The effects of these changes were agonist-selective and had little if any influence on the affinities of the antagonist(s). Taken together, the experimental conditions of binding assays limits the utility of this method for the study of structure-affinity relations of agonists but not of antagonists.

Studies of the natural agonist and its derivatives are best suited to isolated, intact and functional preparations. These systems provide two measures of the agonist-receptor interaction: affinity and relative intrinsic efficacy. Both of these drug properties are chemically independent of one another because they show different relationships to agonist structure (Ruffolo et

al., 1979; Ringdahl and Jenden, 1983) and are therefore useful for receptor characterization and classification. Structure activity studies of 5-HT and some structural analogs were performed on tissues reported to possess 5-HT_2 receptors (table 2). However, most of these studies provided little information about the agonist-receptor interaction; instead, tissue-dependent values such as EC50, intrinsic activity or agonist potency ratios were measured. These kinds of measurements are of limited value. For example, Feniuk et al. (1985) compared the ratios of EC50 values of several tryptamine analogs on the rabbit aorta and the dog saphenous vein. Their results suggested that the 5-HT receptors in these two tissues were different but this hypothesis required definitive corroboration with measures of the affinities of the competitive antagonists ketanserin and spiperone. Agonists, like antagonists, can be used to characterize and classify receptors if their affinities and relative intrinsic efficacies are determined (Leff et al., 1986).

Specific aims.

In this investigation two approaches will be taken to further the pharmacological characterization of 5-HT_2 receptors. First, the interactions of several tryptamine analogs with this receptor will be examined. These experiments will rely on traditional steady-state methods

of analysis to determine drug dissociation constants and relative intrinsic efficacy values. The objective of this work will be to gain some understanding of the structural requirements for recognition at this receptor and activation of this receptor by simple tryptamine analogs. This kind of information would be useful for the development of agonists or antagonists that are selective for 5-HT_2 receptors. Second, the actions of two nonsurmountable antagonists of the response to 5-HT will be studied in an attempt to understand their mechanism of action. Lysergic acid diethylamide (LSD) was reported to be a nonsurmountable antagonist of the response to 5-HT in the isolated rabbit aorta (Black et al., 1983). In this study I will examine the antagonism produced by LSD and by its 2-iodo- derivative (IOL). Steady-state methods will be used to quantify and characterize the interactions of these drugs with the 5-HT_2 receptor. For a more complete analysis of this problem attempts will be made to understand the time-dependent properties of the antagonism produced by LSD and IOL. However, before the kinetics of LSD and IOL are investigated it will be necessary to study, for comparison, the kinetics of simple competitive antagonists like ketanserin, methysergide and spiperone. Insight into the mechanism of action of LSD, IOL and similar antagonists may increase our understanding of drug-receptor interactions.

The rabbit aorta was chosen for this study for three

principal reasons. First, the response to 5-HT is mediated by one receptor previously and properly classified as the 5-HT_2 receptor (Humphrey et al., 1982; Maayani et al., 1984). Second, the response to 5-HT is robust, fade-free and reproducible, necessary characteristics for the quantitative analysis of drug-receptor interactions. Third, tissue-related factors which can complicate drug-receptor studies, e.g., agonist uptake, metabolism and actions at other receptors, can be controlled (Stollak, 1980).

Table 1. Systems in which the response was reported to be mediated by 5-HT_2 receptors.

Response to 5-HT	Species/Tissue	Notes	References
in vitro			
contraction of vascular			
smooth muscle	rabbit aorta	a	Humphrey et al., 1982
		a	Maayani et al., 1984
	rat aorta	b	Cohen et al., 1981
	rat jugular vein	a	Cohen et al., 1981; 1983
	rat portal vein	a	Lemberger et al., 1984
	rat caudal artery	c	Leysen et al., 1982
	human umbilical artery	d	McGrath et al., 1985
	calf coronary artery	e	Kaumann, 1983
	calf pulmonary artery	e	Frenken and Kaumann, 1984

Continued on next page.

Table 1 continued.

contraction of extravascular smooth muscle	rat uterus	f	Ichida et al., 1983
	guinea pig ileum	c	Engel et al., 1984a;b
	guinea pig trachea	c	Van Nueten et al., 1982
PGI_2 formation	bovine aorta cells	a	Coughlin et al. 1984
aggregation	cat platelet	c	De Clerck et al., 1984a;b
	human platelet	c	McBride et al., 1983
		c	Geany et al., 1984
<u>in vivo</u>			
neuronal excitiation	rat brainstem	g	Davies et al., 1985
			Davies and Roberts, 1986
Response to 5-hydroxytryptophan			
wet dog shakes	rat	g	Yap and Taylor, 1983
head twitch	rat	h	Colpaert and Janssen, 1983

Continued on next page.

Table 1 continued.

a. Significant correlation between antagonist dissociation constants determined in binding studies (K_I) and bioassay (K_B).

b. Spiperone pA_2 equaled 9.72.

c. Significant correlation between K_I or IC50 values from binding studies and IC50 values of antagonists of the response.

d. Methysergide pA_2 equaled 8.63..

e. Ketanserin pA_2 was approximately 9.3 and methysergide was a nonsurmountable antagonist.

f. Ketanserin and other 5-HT_2 ligands were potent, nonsurmountable antagonists.

g. Response inhibited by ketanserin and other 5-HT_2 antagonists.

h. Response inhibited by 5-HT_2 antagonists.

Table 2. Agonist structure activity studies in preparations reported to possess 5-HT_2 receptors.

Species/Tissue	Notes	References
Rabbit aorta	a	Feniuk et al., 1985
	b	Leff et al., 1986
Rat aorta	a	Forster and Whalley, 1981
Rat jugular vein	b	Leff et al., 1986
Human Platelets	a	Victorzon et al., 1986
	a	Laubscher and Pletscher, 1979
Rabbit Platelets	a	Graf and Pletscher, 1979
Rat Uterus	c	Gaddum et al., 1955
	d	Bertaccini and Zamboni, 1961
	e	Cerletti et al., 1968

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Table 2 continued.

a. Determined EC50 and intrinsic activity values.

b. Determined K_A and relative intrinsic efficacy values of five tryptamine analogs.

c. Determined inhibitory and stimulatory properties of tryptamines analogs.

d. Determined equipotent molar ratios of tryptamine analogs.

e. Tryptamine analogs tested for their ability to antagonize the effect of 5-HT.

METHODS

Tissue preparation.

The isolated thoracic aorta from male New Zealand White rabbits (1.5-2.2 Kg, Perfection Breeders Douglassville, PA) was used for all experiments. Rabbits were killed by CO_2 asphyxiation and the aortae were removed and trimmed of excess fat and connective tissue. In some experiments the adventitia, a layer of connective tissue that surrounds the aorta, was removed as described by Maxwell et al. (1968). In the process of removing the adventitia some muscle tissue may alo be removed. Rings of tissue, 4 to 6 mm wide, were cut from a single vessel and were suspended between two L-shaped stainless steel hooks mounted in 20 ml organ baths. The baths contained a Krebs buffer of the following composition (mM): NaCl, 110.0; KCl, 4.8; $CaCl_2$, 2.35; $MgSO_4$, 1.20; KH_2PO_4, 1.20; $NaHCO_3$, 25.0; d-glucose, 11.0 and Na_2EDTA, 0.03 in deionized-distilled water. The buffer in each bath was continuously aerated with 5% CO_2 in oxygen, to maintain a pH of 7.5 +/- 0.1, and kept at a temperature of 36.5 +/- 0.5 ^{0}C. Rings of the aorta with adventitia exert a larger force than those without the adventitia. Consequently, tissues with and without the adventitia were initially set to 4 and 2 grams of tension, respectively, and allowed to relax for about

2.5 hr before experimentation. During this time the tissues were exposed to 720 uM iproniazid phosphate (IPN), an irreversible inhibitor of the enzyme monoamine oxidase, for 25 min and then washed twice by overflow at 30 min intervals. IPN was included to block the differential metabolism of the tryptamine analogs (Vane, 1959; Stollak and Furchgott, 1983). Next, the tissues were exposed to 15 uM benextramine tetrahydrochloride monohydrate (BHC), an irreversible antagonist at alpha-adrenergic receptors (Melchiorre et al., 1978), for 30 min and then washed twice at 30 min intervals. BHC eliminated the direct actions of tryptamine (Stollak and Furchgott, 1983) and the potential actions of the other tryptamine analogs at alpha-adrenergic receptors. After the relaxation phase the tension in tissues with and without adventitia was adjusted to 2 and 1 gram, respectively, for all subsequent assays. Before experimentation two cumulative and consecutive concentration-response assays were performed with 5-HT at concentrations of 0.01, 0.1 and 1 uM. These priming assays stabilized the response characteristics of the tissue, e.g., the maximal response and the concentration of agonist which elicits half the maximal response, for subsequent assays. Five minutes before each assay tissues were exposed to 30 uM cocaine hydrochloride to block potential neuronal uptake of the tryptamine analogs (Stollak, 1980). Isometric contractions were measured with Grass FT03C force displacement transducers

attached to a Grass polygraph recorder.

I. Studies with tryptamine analogs.

Analysis of agonist action.

Agonists were administered cumulatively (0.5 $\log_{10}$ unit increments). The actions of all tryptamine analogs were compared to 5-HT. Consecutive assays were usually performed in each tissue as follows: 5-HT, tryptamine analog and 5-HT. This protocol permitted the assessment of spontaneous changes (assumed to be a function of time) in the sensitivity and responsiveness of the tissue to 5-HT. Spontaneous changes in the response to 5-HT were usually small; concentration-response curves (CRCs) from consecutive assays were usually superimposable (fig. 1). Changes in the location parameter (EC50) of the CRC were never larger than 0.3 log units. Changes in the maximal response (responsiveness) were more variable and sometimes showed 10% increases or decreases compared to the first CRC of 5-HT. Due to the reproducibility of the contractile response some assays of tryptamine analogs were not "bracketed" between those of 5-HT; instead a 5-HT assay was followed by one with the test agonist. Three parameters of the CRC were determined from a computer fit of the observed data to the logistic function expressed in the form:

$$E = Emax/(1 + (EC50/[A])^{p}) \quad (1)$$

where E is the observed increase in grams of tension, [A] is the agonist concentration, Emax is the fitted maximum response, EC50 is the [A] at 0.5 Emax, and p is the slope index of the CRC. Relative intrinsic activity (RIA) is defined as follows: Emax T-analog/Emax 5-HT. About 40-50 min were required for a cumulative concentration-response assay. After agonist washout, about 60 min were required for the tissue to relax to basal tone before the start of a second assay.

Antagonist studies.

After an agonist assay, tissues were exposed to one of several concentrations of spiperone for at least one hour and then the agonist assay was repeated in the presence of the antagonist. Spontaneous changes in the parameters of the CRCs of the paired control tissues were negligible. The dissociation constant (K_B) of spiperone was determined with the equation:

$$K_B = [B]/(CR-1) \quad (2)$$

where [B] is the concentration of antagonist and CR is the equieffective concentration ratio.

Two tryptamine analogs, 4-hydroxy-N,N-dimethyltryptamine (psilocin or 4-HDMT) and

4,5-methylenedioxy-N,N-dimethyltryptamine (4,5-MDODMT), were not assayed in the presence of spiperone. Instead, these drugs were used as antagonists of the response to 5-HT. In these particular experiments the antagonists did not elicit a contractile response (the EC50 value of 5-HT was about 0.1 uM). Tissues were exposed to one of several concentrations of the antagonists for 30 to 60 minutes before the 5-HT assay was repeated. Antagonist dissociation constants were determined with the method of Arunlakshana and Schild (1959). When the slope of the Schild plot was not significantly different from one, the pA_2 was obtained from a slope constrained to one (Tallarida et al., 1979).

Determination of agonist dissociation constants (K_A) after receptor inactivation (Method 1).

Method 1 was used to determine the K_A values of some partial agonists and of all agonists with RIA values greater than 0.9. One or two agonists were assayed on a given tissue. Agonist concentration-response assays were performed on untreated tissues to establish the control response parameters (CRC 1). Next, a fraction of the 5-HT_2 receptors was irreversibly inactivated (alkylated) by exposing the tissue to 0.1 uM dibenamine hydrochloride (DB) for 6 to 10 min. The unreacted DB was then washed from the tissue followed by a second wash 15

to 20 min later. Agonist assays were repeated on the treated tissues (CRC 2). CRCs 1 and 2 were then fitted to equation 1. Four agonist concentrations that elicited between 20 to 85% of the maximal response on CRC 2 were chosen and equiactive concentrations were obtained from CRC 1. The K_A values were calculated from a rearranged form (Stollak and Furchgott, 1983) of the equation derived by Furchgott (1966):

$$[A']/[A] = ((1 - q)/qK_A)[A'] + 1/q \qquad (3)$$

where [A] and [A'] are the equiactive agonist concentrations before and after receptor alkylation, and q is the fraction of functional receptors. All data were fit to a least squares regression of [A']/[A] versus [A'] to obtain the values of K_A and q.

Relative intrinsic efficacy (RIE) values were determined according to the procedure of Furchgott and Bursztyn (1967). This method assumes that agonists which elicit equal responses produce equal stimuli (S) such that $S_{5\text{-HT}} = \epsilon_{5\text{-HT}}[AR_{5\text{-HT}}]$ and $S_X = \epsilon_X[AR_X]$ where ϵ is intrinsic efficacy, [AR] is the concentration of the agonist-receptor complex and X is the agonist being compared to 5-HT. Therefore, at any level of equal response $\epsilon_X/\epsilon_{5\text{-HT}} = [AR_{5\text{-HT}}]/[AR_X]$. The fraction of receptors occupied by a given concentration of agonist was determined from the equation:

$$[AR]/[R_T] = [A]/([A] + K_A) \qquad (4)$$

where [A] is the agonist concentration, $[R_T]$ is the total concentration of receptors and K_A is the mean of the dissociation constant obtained from Method 1. Responses to 5-HT and a test agonist, assayed on the same tissue, were expressed as a function of the negative logarithm of $[AR]/[R_T]$. The RIE values of the agonists were determined from the antilog of the interval between equiactive receptor occupancies at the midpoint on the test agonist curve.

Determination of partial agonist dissociation constants (Kp) and RIE values by a comparative multiple-agonist analysis (Method 2).

As most of the tryptamine analogs tested were partial agonists, the method of Barlow et al. (1967), as modified by Kenakin and Black (1978), was used to determine the dissociation constants of partial agonists. This independent analysis provided a check of the results obtained with Method 1. The following equation describes the linear relation between equiactive concentrations of 5-HT and of the partial agonist assayed consecutively on the same tissue:

$$[5\text{-HT}] = K_{5\text{-HT}}G - ([5\text{-HT}]/[P])(1 + G)Kp \quad (5).$$

[5-HT] and [P] are equiactive concentrations of 5-HT and

the partial agonist with dissociation constants of $K_{5\text{-}HT}$ and Kp, respectively, and G is equal to the efficacy term $\epsilon_p/(\epsilon_{5\text{-}HT} - \epsilon_p)$. A plot of [5-HT] vs [5-HT]/[P] is a line with a slope equal to $Kp(1 + G)$ and an intercept equal to $K_{5\text{-}HT}G$. With the $K_{5\text{-}HT}$ value from Method 1 it was possible to obtain direct estimates of Kp:

$$Kp = -\text{slope}/(1 + (\text{intercept}/K_{5\text{-}HT})) \qquad (6)$$

and relative intrinsic efficacy ($\epsilon_p/\epsilon_{5\text{-}HT}$):

$$\epsilon_p/\epsilon_{5\text{-}HT} = 1/(1 + (K_{5\text{-}HT}/\text{intercept})) \qquad (7).$$

In the original formulation of this analysis, Barlow et al. (1967) assumed that a receptor reserve existed for the full agonist and therefore the equiactive concentrations of the full agonist were less than the value of its dissociation constant. Equation 5 does not rely on this assumption (the modification of Kenakin and Black, 1978) and therefore the equiactive concentrations of the full agonist are not necessarily less than the dissociation constant.

II. Agonist and antagonist competition kinetics assays.

A kinetic model for the onset of antagonism.

A general model, describing the interaction of a drug with a receptor, was presented by Furchgott (1964) as:

$$[D]_o \underset{k_{io}}{\overset{k_{oi}}{\rightleftharpoons}} [D]_i + R \underset{k_{-x}}{\overset{k_x}{\rightleftharpoons}} DR.$$

$[D]_o$ and $[D]_i$ are the drug concentrations in the bath and in the biophase, respectively. The "out to in" and "in to out" diffusion rate constants, k_{oi} and k_{io}, are directly proportional to the diffusion coefficient of the drug in the biophase and inversely proportional to the square of the width of the biophase (Rubinow, 1975). This model is described by a set of coupled, nonlinear differential equations which cannot be solved analytically except in two limiting cases. One simplifying assumption is that diffusion through the biophase is the rate limiting step. This assumption leads to the condition that the time course of receptor occupation is determined primarily by k_{io}, the "in to out" diffusion rate constant. The other limiting case is when the drug-receptor interaction is the rate limiting step. The observed rate constant, k_{obs}, of the drug-receptor interaction can then be expressed

(Furchgott, 1964; van Ginneken, 1977) as:

$k_{obs}=k_x(k_{oi}/k_{io})[D]_o+k_{-x}$ (8).

Thus, k_{obs} depends on the rate constants of the drug-receptor interaction, k_x and k_{-x}, and is modified by the partition coefficient, k_{oi}/k_{io}. These approximate solutions of the general model may not be applicable in this system. Furthermore, the added complication of two drugs interacting simultaneously with the receptor necessitated a special experimental design to circumvent these problems.

To obtain information about the kinetics of the interaction between a drug and a receptor the assays were designed so that diffusion was no longer the rate determining step. This was achieved with a high concentration of agonist ([agonist]>>K_A) which ensured that most of the receptors were occupied before the antagonist was added. The rate of the interaction between the antagonist and the receptor depends on the concentration of free, unoccupied receptors; thus in the presence of a high concentration of agonist this rate is greatly decreased. Since the diffusion of the antagonist does not depend on either the concentration of free receptors or on the concentration of the agonist, the rate of the interaction between the antagonist and the receptor becomes the rate limiting step.

The agonist and the antagonist bind to the receptors

in a reversible manner and according to the law of mass action. This is represented by two simultaneous reactions:

$$A + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AR$$

$$B + R \underset{k_4}{\overset{k_3}{\rightleftharpoons}} BR$$

where R is the receptor and A and B are the agonist and the antagonist, respectively. (During an assay it is assumed that the concentrations of A and B, in the bath, do not change significantly.) AR and BR are the drug-receptor complexes. k_1 and k_2 are the reaction rate constants of the agonist; k_3 and k_4 are the reaction rate constants of the antagonist. The differential equations that describe this system are:

$$d[AR]/dt = k_1[A][R] - k_2[AR] \qquad (9)$$

$$d[BR]/dt = k_3[B][R] - k_4[BR] \qquad (10)$$

$$d[R]/dt = k_2[AR] + k_4[BR] - (k_1[A] + k_3[B])[R] \qquad (11).$$

Equation 11 can be eliminated because of the conservation of mass:

$$[R] = R_T - [AR] - [BR] \qquad (12)$$

where R_T is total concentration of receptors.

Equations 9 and 10 constitute a pair of simultaneous differential equations that can be solved with the Laplace transformation. Solving for [AR] we obtain:

$$[AR]/[AR]_0 =$$

$$c/ab-((c-ab)/(b-a))[(e^{-at}/a)-(e^{-bt}/b)] \quad (13).$$

Note that the equation which describes the antagonist-induced decrease in the steady-state concentration of the agonist-receptor complex is biexponential. The relation between the parameters in equation 13 and the kinetic rate constants is defined as follows:

$$a=0.5[D+E+\sqrt{(D+E)^2-4(k_4D+k_2k_3[B])}]$$

$$b=0.5[D+E-\sqrt{(D+E)^2-4(k_4D+k_2k_3[B])}]$$

$$c = Dk_4$$

$$D = k_1[A]+k_2$$

$$E = k_3[B]+k_4.$$

Equation 13 satisfies the boundary conditions of the system. At time zero, $[AR]/[AR]_0 = 1$, the maximal fractional concentration of the agonist-receptor complex. At long times a new steady-state fractional concentration is achieved:

$$[AR]_\infty/[AR]_0 = c/ab =$$

$$([A]+K_A)/([A]+K_A(1+[B]/K_B)).$$

This expression agrees with the one derived from steady state considerations because

$$[AR]_{\infty} = [A]R_T/([A] + K_A(1 + [B]/K_B))$$

and

$$[AR]_O = [A]R_T/([A] + K_A).$$

Experimentally, the antagonist-induced decrease in [AR] is observed as a decrease in the steady-state response to the agonist. Using Stephenson's theory (Stephenson, 1956; Furchgott, 1966) relative measures of [AR] can be extracted from the decrease in the response because the effect (E) is a function (f) of a stimulus (S) produced by the agonist, i.e., $E = f(S)$ and $S = \epsilon[AR]$, where ϵ is the intrinsic efficacy of the agonist. This relation should also apply to nonequilibrium systems if the functional dependence between E and S does not depend on time such that $E_t = f(S_t)$, where $S_t = \epsilon[AR]_t$. The addition of a competitive antagonist does not change ϵ nor does it change f, but it does compete with the agonist for receptors. Therefore, the decrease in the response after the addition of the antagonist is directly proportional to the decrease in [AR].

Design of competition kinetics assays.

The following protocol describes the experimental manipulations performed on a single ring of the aorta. A steady-state response was elicited with either 3 uM 5-HT or 20 uM tryptamine, i.e., 15 or 20 times, respectively, the agonist dissociation constant (Clancy and Maayani, 1985). Antagonist was added to the tissue bath and the decrease in the response was followed until it attained a new steady-state and then both drugs were washed out. Thereafter, the tissue buffer was replaced at 20 min intervals. After 1.5 to 2 hr the assay was repeated with the same concentration of the agonist and a higher concentration of the antagonist; two antagonist concentrations were assayed on the same ring of the aorta. In consecutive assays on a given tissue the steady-state responses to the agonist were approximately the same which indicated tissue viability and the removal of antagonist from the previous assay. In figure 11 the procedure is exemplified by a composite of polygraph tracings of assays performed on one tissue.

Data analysis.

Polygraph tracings of each assay were digitized with the aid of the PROPHET computer system. The digitized points were assigned coordinates of fractional response as

a function of time and were used for parameter estimation and graphical representation. Since equation 13 contains three independent parameters, estimates of only three of the four kinetic rate constants can be obtained. The fourth rate constant can be estimated if the dissociation constant of either the agonist (K_A) or the antagonist (K_B) is known. For example, when K_A is known, k_2/K_A is substituted for k_1 in equation 13 and estimates of k_2, k_3 and k_4 are obtained. Similarly, when K_B is known, k_3K_B is substituted for k_4 in equation 13 and estimates of k_1,k_2 and k_3 are obtained. The equilibrium dissociation constants for 5-HT and spiperone, at 5-HT_2 receptors in the rabbit aorta, are 0.2 uM and 0.6 nM, respectively (Clancy and Maayani, 1985). Parameters were estimated by a weighted, nonlinear, least squares curve fitting procedure, called MKMODEL, available on the PROPHET computer system. The weights were set equal to the size of the observation at any given time to obtain good estimtes of the large rate constants that play an important role at early times. Estimates for the rate constants were optimized by the simultaneous analysis of two assays performed on the same tissue.

Partition coefficients.

The n-octanol/water partition coefficient (log P) of

spiperone was provided by Dr. J.P. Tolleneare of Janssen Pharmaceutica, Beerse, Belgium. Log P values for methysergide and 5-methoxygramine (5-MXG) were determined with a public procedure called LOGP which is available on the PROPHET computer system. LOGP calculates n-octanol/water partition coefficients for molecules based on the method described by Hansch and Leo (1979).

III. Studies of nonsurmountable antagonism.

Antagonism of the response to 5-HT by LSD and IOL.

Cumulative (0.5 $\log_{10}$ unit increments) concentration-response assays for 5-HT were performed on tissues to establish the control response parameters (see equation 1). Tissues were exposed to each concentration of antagonist for 2.5 hr, and 5-HT assays were repeated in the presence of the antagonist. In some experiments (depicted in figs. 16 and 17) three to four assays, in the absence or in the presence of increasing concentrations of antagonist, were performed on each tissue. One tissue served as a paired control, i.e., it was not exposed to the antagonist, to assess spontaneous changes in the control response parameters. In all experiments spontaneous changes in the response parameters of CRCs from the paired control tissues were negligible. In the presence of a competitve or nonsurmountable antagonist the

rightward displacement of the CRC was quantified by measuring the concentration ratio which is the quotient of EC50 values, e.g., EC50+antagonist/EC50-antagonist.

Some experiments indirectly examined the influence of tissue levels of cyclic GMP, the amount of which may be increased by the action of an endothelium-derived relaxation factor (EDRF; Rapoport and Murad, 1983), on the response to 5-HT in the absence and in the presence of 10 nM LSD. In one set of experiments the lumen of some tissues was rubbed with a moistened wooden rod to remove endothelial cells which produce and release the EDRF. In another set of experiments tissues were exposed to 3 uM methylene blue, an inhibitor of soluble guanylate cyclase (Ignarro and Kadowitz, 1985), 30 min before and during concentration-response assays with 5-HT (Alosachie and Godfraind, 1986).

Reduction of the antagonistic effects of LSD and IOL by competitive antagonists.

Each tissue served as its own control. Tissues were exposed to 3 uM 5-HT which elicited a maximal, steady-state response (R_1) and then 5-HT was washed out. Next, tissues were exposed for 2.5 hr to: i) 6 nM LSD or ii) 10 nM IOL or iii) a combination of either LSD or IOL and a competitive antagonist at a concentration of about 50 K_Bs. Finally, the tissues were exposed to

100 uM 5-HT in the presence of the antagonist(s) and a steady-state response was measured (R_2). A higher concentration of 5-HT was used to elicit R_2 in the presence of the antagonist(s). Responses in the presence of the antagonist(s) were expressed as a percent of the original response, e.g., $R_2/R_1 \times 100$.

Decreases in the maximal response to 5-HT by LSD: analysis of the CRC of LSD.

Tissues were exposed to 10 uM 5-HT which elicited a maximal, steady-state response (R_1) and then 5-HT was washed out. Next, tissues were exposed for 2.5 hr to one of three concentrations of LSD in the absence or in the presence of 10 K_Bs of a competitive antagonist. One tissue served as a paired control to account for spontaneous changes in the maximal response to 5-HT. Finally, tissues were exposed to 100 uM 5-HT in the absence or in the presence of the antagonist(s) and a steady-state response was measured (R_2). A higher concentration of 5-HT was used to elicit R_2 in the presence of the antagonist(s). Percent LSD-inhibition of the maximal response was determined as:

$(1 - R_2/R_1) \times 100$ and was plotted as a function of LSD concentration. Equieffective concentration ratios of LSD were determined in the absence and in the presence

of a competitive antagonist. The dissociation constant (K_B) of the competitive antagonist was calculated with equation 2. Spontaneous changes in the maximal response to 5-HT were negligible in the paired control tissues. Therefore, each tissue served as its own control.

Statistics.

All EC50 values and drug dissociation constants are reported as the geometric mean +/- S.E.M. (Gaddum, 1945; Fleming et al., 1972). The standard error of the geometric mean was determined according to the procedure described by De Lean et al. (1982). Unless otherwise indicated, all other averaged measures are the arithmetic mean +/- S.E.M. Analysis of variance or Student's t-test (paired or unpaired as necessary) were used to test for differences in the means of selected measures.

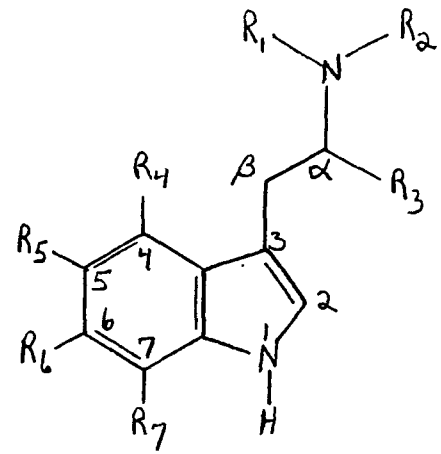
Drugs used.

Solutions of the tryptamine analogs were prepared in deionized-distilled water on the day of the experiment. Drugs (abbreviation) [source] used were as follows: 5-hydroxytryptamine oxalate (5-HT), 5-methoxytryptamine HCl (5-MeOT), 5-methyltryptamine HCl (5-MT), N-w-methyl-5-hydroxytryptamine oxalate (5-HNMT), tryptamine HCl (T), N-methyltryptamine (NMT),

7-methyltryptamine (7-MT) and 6-hydroxytryptamine creatinine sulfate (6-HT) [Sigma Chemical Co., St Louis, MO.]; dl-alpha-methyltryptamine (AMT), benextramine tetrahydrochloride monohydrate (BHC), iproniazid phosphate (IPN), 5-methoxygramine (5-MeOG) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) [Aldrich Chemical Co. Milwaukee, WI]; cocaine HCl, N,N-dimethyltryptamine (DMT) and 4-hydroxy-N,N-dimethyltryptamine (4-HDMT) [National Institute on Drug Abuse, Bethesda MD]; 7-hydroxytryptamine creatinine sulfate monohydrate (7-HT), 4-hydroxytryptamine creatinine sulfate dihydrate (4-HT) and bufotenine (5-HDMT) [National Institute of Mental Health, Rockville, MD]; 4,5-methylenedioxytryptamine (4,5-MDOT) and 4,5-methylenedioxy-N,N-dimethyltryptamine (4,5-MDODMT) [kindly provided by Dr. Toni Kline, Mount Sinai Medical Center, N.Y., NY]; d-lysergic acid diethylamide bitartrate (LSD) [Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD]; dibenamine HCl (DB) [ICN Pharmaceuticals, Plainview, N.Y.]; spiperone and ketanserin tartrate [Janssen Pharmaceuticals, Beerse, Belgium]; methysergide maleate and 2-iodo-d-lysergic acid diethylamide (IOL) [Sandoz Ltd. Basle, Switzerland]; Bay K 8644 (Bayer Inc., Fed. Republic of Germany); methylene blue (Merck, Rahway, NJ). Bay K 8644 was dissolved in absolute ethanol at a concentration of 0.3 mM and stored in the dark at -20 degrees centigrade. 4,5-MDODMT was

synthesized as previously reported (Kline et al., 1982). The structures of 6-HT and 7-HT were confirmed by high resolution nuclear magnetic resonance spectroscopy kindly performed by Michael Angelastro of the Department of Chemistry, S.U.N.Y., Stony Brook. All other chemicals were of analytical grade. Table 3 shows the structures of all the tryptamine analogs used in this study.

Table 3. The structures of the tryptamine analogs used in this investigation.



Drug	R1	R2	R3	R4	R5	R6	R7
5-HT	-H	-H	-H	-H	-OH	-H	-H
5-HNMT	-CH3	-H	-H	-H	-OH	-H	-H
5-HDMT	-CH3	-CH3	-H	-H	-OH	-H	-H
5-MeOT	-H	-H	-H	-H	-OCH3	-H	-H
5-MeODMT	-CH3	-CH3	-H	-H	-OCH3	-H	-H
T	-H	-H	-H	-H	-H	-H	-H

Continued on next page.

Table 3 continued.

Drug	R1	R2	R3	R4	R5	R6	R7
NMT	-CH3	-H	-H	-H	-H	-H	-H
DMT	-CH3	-CH3	-H	-H	-H	-H	-H
4-HT	-H	-H	-H	-OH	-H	-H	-H
4-HDMT	-CH3	-CH3	-H	-OH	-H	-H	-H
4,5-MDOT	-H	-H	-H	-O—CH2—O-		-H	-H
4,5-MDODMT	-CH3	-CH3	-H	-O—CH2—O-		-H	-H
5-MT	-H	-H	-H	-H	-CH3	-H	-H
7-MT	-H	-H	-H	-H	-H	-H	-CH3
AMT	-H	-H	-CH3	-H	-H	-H	-H
6-HT	-H	-H	-H	-H	-H	-OH	-H
7-HT	-H	-H	-H	-H	-H	-H	-OH

RESULTS

I. Studies with tryptamine analogs.

5-HT induced a graded-contractile response in the concentration range of 0.01 to 3 uM. The results of a representative experiment with 5-HT and Bufotenine (5-HDMT), assayed on a single tissue, are displayed in figure 1 and they show that 5-HDMT is a partial agonist. The tracings in figure 1A illustrate the order in which the two agonists were assayed. The steady-state response elicited by each concentration of agonist was used to construct the CRCs in figure 1B. Table 4 summarizes the results of similar experiments with the other tryptamine analogs. Thirteen tryptamine analogs were partial agonists with relative intrinsic activity (RIA) values ranging from 0.06 for 6-HT to 0.89 for tryptamine. Three other drugs, 5-methoxytryptamine (5-MeOT), 4-HT and 4,5-MDOT, had RIA values greater than 0.9 and appeared to be full agonists. The agonist EC50 (nM) values ranged from 44 for 5-MeOT to 1525 for alpha-methyltryptamine (AMT). The mean slope indices of the agonist CRCs were similar and ranged from 1.35 to 1.78. 6-HT and 7-HT were the least active agonists; when assayed at 10 uM these drugs did not usually elicit a measureable response. At a concentration of 100 uM, 6-HT and 7-HT elicited responses in three of seven and all of five experiments,

respectively. Neither the EC50 nor the RIA values of 6-HT and 7-HT could be determined. However, the fractional responses at 100 uM 6-HT and 7-HT are included in table 4.

To determine if the agonist-induced response was mediated by the 5-HT_2 receptor, the K_B of spiperone was determined with each agonist except 6-HT, 7-HT, 4-HDMT and 4,5-MDODMT, due to their low RIA values. The responses to AMT in the absence and presence of spiperone are presented in figure 2. The pK_B values of spiperone that were determined with each agonist are summarized in table 5; these values are similar and range from 8.97 to 9.39. For each agonist, spiperone fulfilled the criteria of a competitive antagonist, i.e., the antagonism was surmountable and reversible and the agonist CRCs were displaced to the right in a parallel manner.

Dissociation constants (K_A) of agonists determined with Method 1.

In figure 3A 5-HT and 5-HDMT were assayed on the same tissue before and after receptor alkylation with dibenamine (DB). Analyses of the data with Method 1 are shown in figure 3B. In this experiment the fractions of functional receptors (q) after DB were similar for both agonists. This result and those shown in table 5 suggested that the contractile responses to 5-HT and 5-HDMT were mediated by the 5-HT_2 receptor. For each of the

agonists assayed there were no significant differences in the slope indices of their CRCs before and after DB (t-tests; $0.10<p<0.86$). Figure 4 compares the occupancy-response curves of 5-HT and 5-HDMT assayed on the same tissues. The antilog of the equiactive interval between these two curves is a measure of the RIE. The K_A and RIE values (Method 1) of most of the agonists are summarized in columns two and three of table 6. The RIE values of 5-HT and 5-MeOT (t-Test; $p>0.3$) and of 5-HT and 4,5-MDOT (t-Test; $p>0.2$) were not significantly different. However, the RIE values of 5-HT and 4-HT were significantly different ($p<0.05$). The remaining partial agonists had RIE values significantly less than one.

Method 1 is based upon several assumptions, two being that irreversible antagonism of the response to an agonist occurs as a consequence of receptor alkylation and the receptor population is uniform with respect to affinity (agonist and antagonist) and intrinsic efficacy (agonist) before and after alkylation (Besse and Furchgott, 1976). In one experiment, pre-exposure of the tissue to 25 nM spiperone for 45 min prevented DB (0.1 uM for 8 min) from inactivating the response to 5-HT. In a paired control ring not exposed to spiperone the maximal response to 5-HT was decreased by 64% after DB (0.1 uM for 8 min). After the removal of spiperone and DB the CRC of 5-HT was superimposable on the original CRC in that tissue (not shown). These results suggested that DB inactivated the

receptor and not a component of the effector system. The antagonism produced by spiperone was examined on tissues pretreated with DB (0.1 uM for 8 min). On pretreated tissues the mean pKB of spiperone was 9.20 +/- 0.08 (n=2), consistent with the values obtained on untreated tissues (table 5). These results show that after DB decreased the maximal response to 5-HT by 60 (n=1) and 90 (n=1) percent the remaining receptors had the same affinty for spiperone as the total population of 5-HT receptors.

Dissociation constants (Kp) and RIE values of partial agonists determined with Method 2.

The results of a representative Barlow plot to determine the Kp and RIE values of 5-HDMT are shown in figure 5. The Kp and RIE values for most of the partial agonists tested are summarized in columns four and five of table 6. As shown in table 6 some agonists (5-HNMT, 5-HDMT, T, 5-MT and AMT) were examined with both Methods 1 and 2 and a correlation between the Kp and K_A values was statistically significant ($r = 0.98$; $0.002<p<0.005$), indicating that these two sets of data were similar. The RIE values from both methods correlated less well ($r = 0.86$; $0.05<p<0.1$) due primarily to 5MT.

Dissociation constants of tryptamine analogs used as antagonists of the response to 5-HT.

6-HT and 7-HT were tested as antagonists of the response to 5-HT to determine their affinities for the 5-HT_2 receptor. 7-HT (two experiments) at concentrations of 1 to 100 uM did not antagonize the response to 5-HT (not shown). The antagonism produced by 6-HT (three experiments) was nonsurmountable. Compared to a paired control ring, 10 uM 6-HT had no observable influence on the response to 5-HT. However, 6-HT at concentrations of 30 and 100 uM displaced the CRC of 5-HT to the right in a concentration-dependent manner and decreased the maximal response (not shown). The dissociation constant of 6-HT was not determined because it did not appear to act as a simple competitive antagonist.

Aside from 6-HT and 7-HT, 4-HDMT and 4,5-MDODMT were the weakest agonists in this study. It should be noted that 4-HDMT and 4,5-MDODMT did not elicit contractile responses unless the tissue was pharmacologically sensitive, defined by a 5-HT EC50 of about 50 nM. The responses to 4-HDMT and 4,5-MDODMT, compared to 5-HT, are shown in figures 6 and 7, respectively . Due to their low RIE values both drugs were tested as antagonists of the response to 5-HT. As shown in figures 8 and 9 4-HDMT and 4,5-MDODMT, respectively, behaved as competitive

antagonists of the response. The antagonist dissociation constants of 4-HDMT and 4,5-MDODMT (from fig. 8 and 9) are 57 and 15 nM , respectively, smaller than but consistent with the values obtained in column four of table 6.

The relation between response and occupancy.

The EC50 values of most of the agonists are less than or equal to their dissociation constants listed in table 6. Generally, the ratio KA:EC50 is directly related to agonist intrinsic efficacy. Figure 10 is a simulation of percent agonist response versus percent receptor occupancy for the agonists, 5-HT, T and BUF. The percent agonist response was determined with equation 1 using the EC50 and slope values from table 4 and setting each agonist maximal response equal to 100 percent. Similarly, receptor occupancy was determined with equation 4 and the agonist dissociation constants (Method 1) in table 6. It is assumed that the relation between agonist concentration and receptor occupancy is described by a rectangular hyperbola. In figure 10 the relation between response and occupancy appears sigmoidal. This simulation shows that about 17% of the receptors were occupied by 5-HT at one half its maximal response. In addition, half-maximal responses for the partial agonists T and 5-HDMT occurred when about 26 and 35% of the receptors were occupied, respectively. In contrast to the other agonists, the EC50

values of 4-HDMT and 4,5-MDODMT were greater than their agonist and antagonist dissociation constants in table 6; this interesting observation is accounted for in the discussion.

Structure-activity observations.

Table 6 lists the dissociation constants and RIE values of the drugs used in this study. Five sets of tryptamine analogs are indicated with brackets, each differing by the nature or location of the substituents on the indole ring system. Within each set the tryptamine analogs differ by the absence or the presence of one or two methyl groups on the side-chain nitrogen. Within each set of agonists RIE values decreased significantly with the addition of one or two methyl groups to the side chain nitrogen; this effect was most evident for analogs with substituents at position four on the indole ring. 4-HT and 4,5-MDOT were among the most efficacious drugs in table 6 but their dimethylated derivatives had the lowest RIE values.

Among the first three sets of drugs in table 6 the dissociation constants (determined with Methods 1 and/or 2) were similar within each group. There was at most a two-fold difference between the dissociation constants of DMT and those of NMT and tryptamine (Method 2). In contrast to the first three sets of agonists the

dissociation constants of the tryptamine analogs with substituents at the four position showed the largest apparent differences. In both sets of 4-substituted drugs the dissociation constants of the dimethylated derivatives (determined with Method 2) were about 4.5 times smaller than those of their respective primary amine analogs (determined with Method 1). This discrepancy was substantiated and enhanced by measures of the antagonist dissociation constants of the dimethylated analogs.

II. Results of competition kinetics studies.

The steady-state response to an agonist.

The response of the rabbit aorta to 3 uM 5-HT or 20 uM tryptamine showed little spontaneous decay over the time required to measure the onset of antagonism (fig. 11). The spontaneous decay of the response in control assays was not used to "correct" for the effect of time in assays with the antagonist. First, the time required for the completion of an antagonist assay was much shorter than the time for the spontaneous decay to reach one-half the maximal response. For example, most of the decrease in the response after the addition of the antagonist occured within the first 10 min of the assay whereas the spontaneous decay in control assays diminished the response by less than 10% in this time. Second, the

spontaneous decay may be due to receptor desensitization which depends on the fraction of receptors occupied by the agonist (Cory et al., 1986). The fraction of receptors occupied by the agonist is constant in the control assay but decreases rapidly after the antagonist is added. Therefore, correcting for the spontaneous decay of the response in control assays would be invalid.

Competition kinetics in the absence of the adventitia.

Estimates of k_2, the dissocciation rate constant of the agonist, and of k_3 and k_4, the rate constants of the antagonist, were obtained from kinetic assays performed with 3 uM 5-HT. The concentrations (nM) of the potent antagonists were 20 to 600 for spiperone, 50 to 1000 for ketanserin and 20 to 400 for methysergide. A representative experiment with 5-HT and spiperone is shown in figure 12. Table 7 summarizes the results of assays with spiperone, ketanserin and methysergide together with the antagonist dissociation rate constants measured in high affinity binding experiments by Leysen and Gommeren (1986). There was good agreement between the antagonist dissociation rate constants obtained in the isolated rabbit aorta and in the binding experiments. The average values of k_2 obtained with each of the high affinity antagonists were not statistically different from each other (ANOVA;p=0.706). The average values of pK_B,

calculated as $-\log[k_4/k_3]$, for these three antagonists were in good agreement with steady-state pK_D values determined in the isolated rabbit aorta (see table 7).

In other experiments we examined the kinetics of competition between 20 uM tryptamine and 10 to 600 nM spiperone. The equilibrium K_B of spiperone was used in the analysis of these experiments. With this information the kinetic analysis yielded parameter estimates for k_1 and k_2, the rate constants of the agonist, and k_3, the association rate constant of the antagonist. The pK_A of tryptamine, calculated as $-\log[k_2/k_1]$, was 5.57 +/- 0.10, in good agreement with the steady-state pK_D determined in the rabbit aorta (Clancy and Maayani, 1985) as listed in table 7.

If antagonist diffusion is not rate limiting then estimates for k_3 and k_4 should be related to the affinity of the antagonist for the receptor. Spiperone, ketanserin and methysergide are high affinity, competitive antagonists at 5-HT_2 receptors and have dissociation constants of about 1 nM (Apperley et al., 1976; Humphrey et al., 1982; Maayani et al. 1984). Table 7 shows that the rate constants for these three antagonists are similar. However, experiments with a low affinity antagonist, such as 5-MeOG, should test the sensitivity of the kinetic analysis to the pharmacological potency of the antagonist. The results of a representative experiment

with 5-HT and 5-MeOG are shown in figure 13 and table 7 summarizes the experiments with 5-MeOG. The average value of k_3 for 5-MeOG was 0.87 +/- 0.11 $(uMmin)^{-1}$, significantly smaller than the average values of k_3 estimated for the more potent antagonists (see table 7). In addition the average value of k_4 for 5-MeOG was 0.31 +/- 0.08 min^{-1}, significantly larger than the average values of k_4 estimated for the more potent antagonists. The pK_B for 5-MeOG, calculated as $-\log[k_4/k_3]$, was 6.49 +/- 0.05, in good agreement with the pK_D determined in steady-state experiments. For example, 5-MeOG displaced the CRC of 5-HT in a dextral, parallel manner without decreasing the maximal response (n=2). A Schild plot for 5-MeOG had a slope of 1.01 (0.94-1.08) and a pA_2 (listed under pK_D in table 7) of 6.21 +/- 0.03, i.e., a K_B of 0.62 uM. This dissociation constant is 2.3 times smaller than the one reported by Stollak and Furchgott (1983). In experiments with 5-MeOG the average value of k_2, the dissociaion rate constant of 5-HT, was almost two times larger than the values obtained with the high affinity antagonists.

The decrease in the response to 5-HT after the addition of 2 to 30 uM of 5-MeOG appeared monophasic, e.g., see figure 13. However, with higher concentrations of 5-MeOG the decrease was biphasic and consisted of a rapid decline in the response followed by a slower

decline. The concentrations of 5-MeOG used in the experiments summarized in table 7 varied from 2.5 to 20 uM.

Competition kinetics in the presence of the adventitia.

Assays were performed with intact adventitia to examine further the influence of diffusion on the kinetics of drug competition. 3 uM 5-HT was used in all assays and estimates of k_2, k_3 and k_4 were obtained. Concentrations (nM) of the high affinity antagonists were 15 to 400 for spiperone, 40 to 500 for ketanserin and 75 to 400 for methysergide. Figure 14 shows a representative experiment with 5-HT and spiperone. A visual comparison of the kinetics of equal concentrations of spiperone in the absence (fig. 12, curve A) and in the presence of the adventitia (fig. 14, curve A) showed that the adventitia did not influence the antagonist-induced decrease in the response. Similarly, the rates of action of methysergide and ketanserin were not different in the absence or in the presence of the adventitia, as shown by the similarities in the values of k_3 and k_4 for these three antagonist in tables 7 and 8. The average pK_B values, calculated as $-\log[k_4/k_3]$, for each of the high affinity antagonists agreed with the steady-state determinations, pK_D, listed in table 7. Average

values of k_2, the dissociation rate constant of 5-HT, obtained with each of the high affinity antagonists were not statistically different from each other (ANOVA; p = 0.651). However, the average value of k_2 obtained from assays performed in the absence of the adventitia was somewhat larger than that obtained in the presence of the adventitia. The difference was less than two-fold and an analysis of variance showed that there was no statistical difference between these sets of values (ANOVA; p = 0.174).

In contrast to the high affinity antagonists, the rate of action of 5-MeOG decreased in assays performed in the presence of the adventitia. A representative experiment with 5-HT and 5-MeOG is shown in figure 15. A visual comparison of the kinetics of equal concentrations of 5-MeOG in the absence (fig. 13, curve A) and in the presence of the adventitia (fig. 15, curve A) showed that the adventitia had a small effect on the kinetics of the assay. The rate constants for 5-MeOG, k_3 and k_4, summarized in table 8 supported this observation. In the presence of the adventitia both the association and the dissociation rate constants decreased by the same factor. Thus, the pK_B for 5-MeOG obtained in the absence or in the presence of the adventitia remained the same. The average value of k_2, the dissociation rate constant of 5-HT, was 0.68 +/- 0.18 min^{-1}, not statistically different from that obtained in assays performed in the

absence of the adventitia (t-test; p = 0.964).

In the presence of the adventitia the decrease in the 5-HT response after the addition of 10 to 100 uM of 5-MeOG appeared monophasic. The 5-MeOG concentrations used for the experiments summarized in table 8 were 10 and 30 uM.

III. Results of studies with LSD and IOL.

Antagonism of the response to 5-HT by LSD and IOL.

LSD and IOL were potent antagonists of the response to 5-HT. As shown in figure 16 the presence of either 6 nM LSD or 12.5 nM IOL reduces the maximal response and shifts the CRC to the right in a nonparallel manner. The antagonism produced by LSD and IOL was selective for the response to 5-HT. CRCs of histamine (n=2), phenylephrine (n=1) and KCl (n=1) were not affected by the presence of 6 nM LSD (not shown). Similarly, CRCs of histamine (n=3) and KCl (n=2) were not affected by the presence of 50 nM IOL (not shown). The removal of endothelial cells (n=2) or the presence of 3 uM methylene blue (n=2) did not influence the CRC of 5-HT in the absence or in the presence of 10 nM LSD (not shown).

The effects of spiperone on the steady-state properties of the antagonism produced by LSD and IOL are also shown in figure 16. In the presence of spiperone and either of the nonsurmountable antagonists the maximal

response to 5-HT was increased compared to that obtained in the presence of only the nonsurmountable antagonist. In addition, the CRCs were further displaced to the right and they were essentially parallel to the control curves. The antagonist effects of LSD and IOL were slowly reversed (over three to four hours) by washing the tissue with buffer.

The rightward shift of the CRC by both LSD and IOL suggests that competitive antagonism may be a component of the overall antagonist effects. IOL decreased the maximal response to 5-HT less than did LSD. Therefore, the antagonism produced by IOL was studied in an attempt to quantify the competitive component of the antagonism. In the presence of 2.5, 12.5 and 50 nM IOL the CRC of 5-HT was displaced to the right in an antagonist-concentration dependent manner (fig. 17). Each concentration of IOL decreased the maximal response by about 30%. In addition, the slope indices of the CRC (p in equation 1) decreased with increasing concentrations of IOL.

Both IOL (Engel et al., 1984b; Kadan et al., 1984) and spiperone (Peroutka and Snyder, 1979; Leysen et al., 1982) bind to 5-HT_2 binding sites with high affinity. Therefore, their antagonism of the response, i.e., the rightward displacements of the CRC, should be additive as shown by the results summarized in table 9.

Reduction of the antagonistic effects of LSD and IOL by competitive antagonists.

The results above suggest that spiperone can protect the maximal response to 5-HT by binding to the 5-HT_2 receptor. This hypothesis was further tested with the competitive antagonists ketanserin, methysergide and 5-methoxygramine (5-MeOG). These 5-HT_2 antagonists are structurally different and their dissociation constants (nM) range from about 1 (ketanserin and methysergide; Humphrey et al., 1982) to 600 (5-MeOG; see above). In these experiments single concentrations of 5-HT were assayed (see methods). The magnitude of the response to 5-HT and the time to half of the maximal response (t1/2) were determined in the absence and in the presence of the antagonist(s). In the presence of 6 nM LSD or 10 nM IOL (table 10) the values of t1/2 increased and the maximal response to 5-HT decreased. When tissues were simultaneously exposed to a nonsurmountable and about 50 K_Bs of a competitve antagonist the values of t1/2 and of the maximal response were similar to those obtained in control assays. The extent of the protection obtained with each competitve antagonist was similar. In the presence of only a competitive antagonist the t1/2 and maximal response values were similar to those of the control.

Decreases in the maximal response to 5-HT by LSD: analysis of the CRC of LSD.

Since 6 nM LSD decreased the maximal response to 5-HT to about 20 percent of the control value (fig. 16 and table 10) it was possible to study the concentration-effect relation of LSD. These experiments were conducted to quantify the nonsurmountable component of the antagonism produced by LSD. As shown in figure 18 the LSD-induced decrease in the maximal response to 5-HT is concentration dependent from 0.1 to 1 nM. This effect was antagonized by 10 K_B of ketanserin and 5-MeOG. In the presence of either antagonist the CRC of LSD was displacd to the right by one order of magnitude. The dissociation constants of ketanserin (n=2) and 5-MeOG (n=2) were 1.17 +/- 0.13 nM and 0.58 +/- 0.08 uM, respectively.

Competition kinetics assays.

The onset of antagonism produced by LSD and IOL was examined and compared to that of methysergide, a structurally similar competitive antagonist. A representative experiment with methysergide is shown in figure 19 in which the decrease in the steady-state response appears first-order. As shown in figures 20 and 21 the onset of the antagonism produced by LSD (n=3) and

IOL (n=3) appeared sigmoidal and the data were not adequately described by the competition kinetics model (equation 13), e.g., some of the kinetic rate constants determined in experiments with LSD or IOL were negative values.

LSD and IOL in the presence of Bay K 8644.

The agonist properties of LSD and IOL were studied in tissues exposed to 300 nM Bay K 8644. 10 nM IOL (about 10 K_Ds, Engel et al., 1984b) did not elicit a contractile response (n=2). However, 20 nM LSD (about 10 K_Ds, Leysen et al., (1982)) contracted the tissue with a RIA value of 0.52 +/- 0.12 (n=4). Figure 22 shows representative tracings of LSD activity. The contractile response to LSD was inhibited by prior exposure (30 min) of the tissue to 200 nM spiperone (n=3) (fig. 22A). A competition kinetics assay was performed with LSD acting as the agonist (fig. 22B). The addition of 200 nM spiperone had little or no effect on the steady-state response elicited by 20 nM LSD (n=3). This observation differed from those obtained with a simple agonist/antagonist pair as shown in figures 12,13,14,15 and 19.

Table 4. Parameters of the concentration-response curves of the tryptamine analogs assayed on the isolated rabbit aorta.

Drug	EC50 (nM)[a]	Slope[b]	RIA[b,c]	N[d]
5-HT	57+/-4	1.63+/-0.02	1	41
5-HNMT	120+/-16	1.72+/-0.09	0.78+/-0.03	6
5-HDMT	116+/-13	1.63+/-0.02	0.57+/-0.04	9
5-MeOT	44+/-13	1.65+/-0.04	0.98+/-0.01	4
5-MeODMT	111+/-8	1.47+/-0.06	0.60+/-0.04	6
T	566+/- 76	1.65+/-0.05	0.89+/-0.01	5
NMT	1070+/-210	1.78+/-0.17	0.43+/-0.13	4
DMT	502+/-125	1.74+/-0.07	0.33+/-0.06	5
4-HT	269+/-73	1.55+/-0.08	0.93+/-0.02	5
4-HDMT	233+/-11	1.35+/-0.18	0.19+/-0.03	3
4,5-MDOT	57+/-25	1.59+/-0.07	0.94+/-0.02	5
4,5-MDODMT	84+/-8	1.36+/-0.04	0.15+/-0.08	3

Continued on next page.

Table 4 continued.

Drug	EC50 (nM)	Slope	RIA	N
5-MT	286+/-36	1.64+/-0.07	0.88+/-0.02	5
7-MT	1040+/-140	1.72+/-0.05	0.75+/-0.04	3
AMT	1525+/-315	1.50+/-0.09	0.71+/-0.06	5
6-HT	N.D.[e]	N.D.	0.06+/-0.05[f]	7
7-HT	N.D.	N.D.	0.13+/-0.05[f]	5

[a] Values are the geometric mean +/- S.E.M.

[b] Values are the arithmetic mean +/- S.E.M.

[c] Relative intrinsic activity.

[d] Number of rabbits used.

[e] Not determined.

[f] Fractional response at 100 uM.

Table 5. Dissociation constants of spiperone determined with several tryptamine analogs in the isolated rabbit aorta.

Drug	pK_B[a]	N[b]
5-HT	9.21+/-0.11	4
5-HNMT	9.08+/-0.05	2
5-HDMT	9.16+/-0.15	2
5-MeOT	8.97	1
5-MeODMT	9.14+/-0.14	2
T	9.23	1
NMT	9.24+/-0.08	3
DMT	9.15	1
4-HT	9.24+/-0.09	2
4-HDMT	N.D.	
4,5-MDOT	9.27+/-0.01	2

Continued on next page.

Table 5 continued.

Drug	pK_B[a]	N[b]
4,5-MDODMT	N.D.	
5-MT	9.32	1
7-MT	9.28+/-0.02	2
AMT	9.39	1
6-HT	N.D.[c]	
7-HT	N.D.[c]	

[a] The negative logarithm of the dissociation constant.

[b] Number experiments.

[c] Not determined.

Table 6. Dissociation constants and relative intrinsic efficacy values of the tryptamine analogs at 5-HT2 receptors in the isolated rabbit aorta.

Drug	K_A (nM)	RIE (Method 1)	K_P (nM)	RIE (Method 2)
5-HT	250+/-20 (7)	1.0	---	1.
5-HNMT	210+/-40 (5)	0.25+/-0.03 (6)	180+/-30 (3)	0.24+/-0.03 (3)
5-HDMT	220+/-40 (3)	0.28+/-0.02 (9)	110+/-20 (4)	0.20+/-0.01 (4)
5-MeOT	140+/-40 (5)	0.86+/-0.12 (3)	---	---
5-MeODMT	---	---	125+/-10 (6)	0.21+/-0.02 (6)
T	1600+/-460 (4)	0.61+/-0.09 (5)	1090+/-70 (5)	0.48+/-0.06 (5)
NMT	---	---	1060+/-170 (4)	0.17+/-0.02 (4)
DMT	---	---	506+/-167 (5)	0.16+/-0.02 (5)
4-HT	875+/-260 (4)	0.80+/-0.06 (5)	---	---
4-HDMT	---	---	146+/-38 (3)[a]	0.08+/-0.01 (3)
4,5-MDOT	276+/-11 (4)	1.21+/-0.20 (5)	---	---
4,5-MDODMT	---	---	50+/-8 (3)[b]	0.07+/-0.02 (3)

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Table 6 continued.

Drug	K_A	RIE (Method 1)	K_P	RIE (Method 2)
5-MT	1300+/-320 (4)	0.61+/-0.08 (5)	530+/-80 (4)	0.34+/-0.07 (4)
7-MT	---	---	1280+/-220 (3)	0.35+/-0.07 (3)
AMT	3900+/-1150 (3)	0.41+/-0.05 (5)	1900+/-200 (4)	0.25+/-0.03 (4)
6-HT	---	---	---	---
7-HT	---	---	---	---

Values for the dissociation constants represent the geometric mean +/- the S.E.M. Values for relative intrinsic efficacy represent the arithmetic mean +/- the S.E.M. The number of individual experiments is indicated within parentheses.

[a] The pA_2 (-log of the antagonist dissociation constant) of 4-HDMT is 7.25 (7.23-7.27), i.e., a K_B of 57 nM.

[b] The pA_2 of 4,5-MDODMT is 7.82 (7.69-7.95), i.e., a K_B of 15 nM.

Table 7. Estimated rate constants for 5-HT, T and some 5-HT_2 antagonists in the isolated rabbit aorta. The kinetics assays were performed in the absence of the adventitia. All values are the mean +/- S.E.M. The numbers in parentheses are the number of experiments performed.

Agonist	Antagonist	k_1 $(uMmin)^{-1}$	k_2 min^{-1}	k_3 $(uMmin)^{-1}$
5-HT	Spiperone (4)	1.76+/-0.18[a]	0.35+/-0.03	90.77+/-17.61
T	Spiperone (4)	0.14+/-0.01	0.39+/-0.09	50.80+/-15.10
5-HT	Methysergide (3)	2.11+/-0.52[a]	0.42+/-0.10	43.27+/-13.35
5-HT	Ketanserin (3)	1.85+/-0.16[a]	0.37+/-0.03	36.57+/-6.86
5-HT	5-MeOG (4)	3.37+/-0.73[a]	0.67+/-0.14	0.87+/-0.11

Continued on next page.

Table 7 continued.

Agonist	Antagonist	k_4 min	k_{-X}[b] min	$p(k_{-X}/k_X)$[c]	$p(K_D)$[d]
5-HT	Spiperone (4)	0.04+/-0.01	0.095	9.44+/-0.22	9.22[e]
T	Spiperone (4)	---	---	5.57+/-0.10	5.74[e]
5-HT	Methysergide (3)	0.07+/-0.02	0.04	8.76+/-0.09	8.49[f]
5-HT	Ketanserin (3)	0.05+/-0.03	0.12	9.04+/-0.25	8.72[g]
5-HT	5-MeOG (4)	0.31+/-0.08	---	6.49+/-0.05	6.21[h]

a) Calculated for 5-HT from $k_1 = k_2/K_A$.

b) Dissociation rate constant of the antagonist determined in high affinity binding studies (Leysen annd Gommeren, 1986).

c) Negative logarithm of the calculated dissociation constant.

d) Negative logarithm of the equilibrium dissociation constant.

e) Clancy and Maayani (1985).

f) Apperley et al. (1976).

g) Humphrey et al. (1982).

h) Reported in this study.

Table 8. Estimated rate constants for 5-HT and some 5-HT_2 antagonists in isolated rabbit aorta. The kinetics assays performed in the presence of the adventitia. All values are the mean +/- S.E.M. The numbers in parentheses are the number of experiments performed.

Antagonist	k_a min^{-1}	k_3 $(uMmin)^{-1}$	k_4 min^{-1}	$p(k_{-X}/k_X)$
Spiperone (2)	0.29+/-0.03	146.00+/-2.00	0.032+/-0.002	9.65±0.04
Methysergide (3)	0.23+/-0.04	36.60+/-8.10	0.05+/-0.01	8.82±0.13
Ketanserin (3)	0.24+/-0.04	59.00+/-21.80	0.06+/-0.01	8.91±0.20
5-MeOG (4)	0.68+/-0.18	0.19+/-0.04	0.07+/-0.01	6.42±0.10

Table 9. Combined antagonism of the response to 5-HT produced by IOL and spiperone in the isolated rabbit aorta. Listed are the means of concentration ratios (CR) +/- S.E.M. for 5-HT (n=3).

	spiperone (10 nM)	IOL (10 nM)	spiperone+IOL
observed CR[a]	16 +/-1	26 +/-3	41 +/-2[b]
expected CR[c] (same receptor)	18	12	29[b]
expected CR[d] (different receptors)	--	--	216
Response maximum (% of control)	100 +/0	57 +/-10	73 +/-3

Continued on next page.

Table 9 continued.

a. Determined from the EC50 values of CRCs of 5-HT in the absence and in the presence of antagonist(s).

b. Calculated from observed $CR_{(spip)} + CR_{(IOL)} - 1$ (Paton and Rang, 1965).

c. For two antagonists binding to the 5-HT_2 receptor. Determined from the relation: $CR = 1 + [B]/K_B$. The K_B of spiperone is 0.6 nM (Clancy and Maayani, 1985). The K_D of IOL at 5-HT_2 binding sites is 0.91 nM (Engel et al., 1984b).

d. Expected if IOL does not bind to the agonist-recognition site on the 5-HT_2 receptor. Calculated from $CR_{(spip)} \times CR_{(IOL)}$ (Paton and Rang, 1965).

Table 10. The nonsurmountable antagonist effect of 6 nM LSD and 10 nM IOL: protection of the maximal response to 5-HT by some 5-HT_2 antagonists in the rabbit aorta. Values shown are the mean +/- S.E.M.(n=2).

[5-HT](uM)	[Antagonist(s)](uM)	Maximal Response[a] (% of Control)	t1/2 (min)[a]
3	---	100	0.5+/-0
100	LSD	17.6+/-4.6	9.4+/-2.9
100	LSD+0.2 methysergide[b]	93.5+/-6.5	1.25+/-0.25
100	LSD+0.1 ketanserin[c]	87.5+/-6.5	1.0+/-0
100	LSD+30 5-MeOG[d]	89.0+/-11.0	0.75+/-0.25

Continued on next page.

Table 10 continued.

[5-HT] (uM)	[Antagonist(s)] (uM)	Maximal Response[a] (% of Control)	t1/2 (min)[a]
3	---	100	0.53+/-0.08
100	IOL	81+/-3	7.5+/-1.0
100	IOL+0.2 methysergide	105+/-3	1.5+/-0.5
100	IOL+0.1 ketanserin	97.5+/-5.5	1.5+/-0.5
100	IOL+30 5-MeOG	104.5+/-4.5	0.75+/-0.25

a. In the presence of the competitive antagonists, the values were similar to the control.

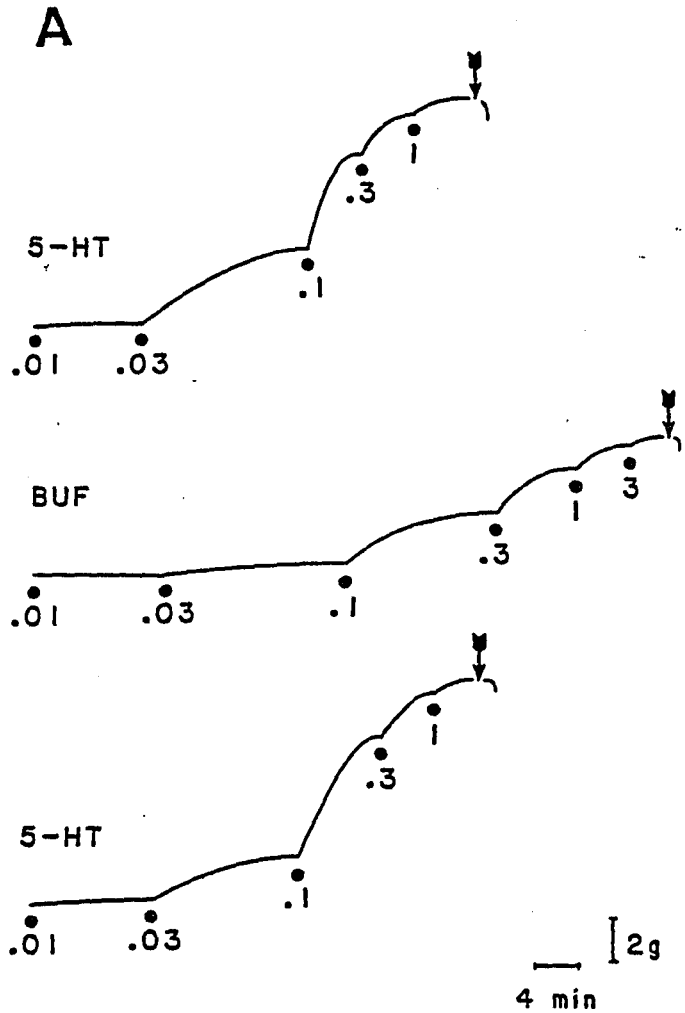
b. The dissociation constant of methysergide was reported to be 3.2 nM (Apperley et al., 1976).

c. The dissociation constant of ketanserin was reported to be 2 nM (Humphrey et al., 1982).

d. The dissociation constant of 5-MeOG was reported to be 0.6 uM (see table 7).

Figure 1. Contractile responses to 5-HT and Bufotenine (BUF or 5-HDMT) in the isolated rabbit aorta. A. Representative tracings of cumulative concentration-response assays for 5-HT and 5-HDMT on the same tissue. The dots denote the time of agonist (uM) addition. The arrows denote the time of agonist washout. B. Concentration-response curves of 5-HT and 5-HDMT obtained from the tracings in panel A. The curves were obtained from a fit of the data to equation 1, yielding the following results:

Agonist	symbol	EC50 (nM)	Emax (g)	p
5-HT	○	42.	9.8	1.82
5-HDMT	△	120.	6.5	1.47
5-HT	□	56.	10.2	1.97


A
5-HT
.01
.03
.1
.3
1
BUF
.01
.03
.1
.3
1
3
5-HT
.01
.03
.1
.3
1
2g
4 min

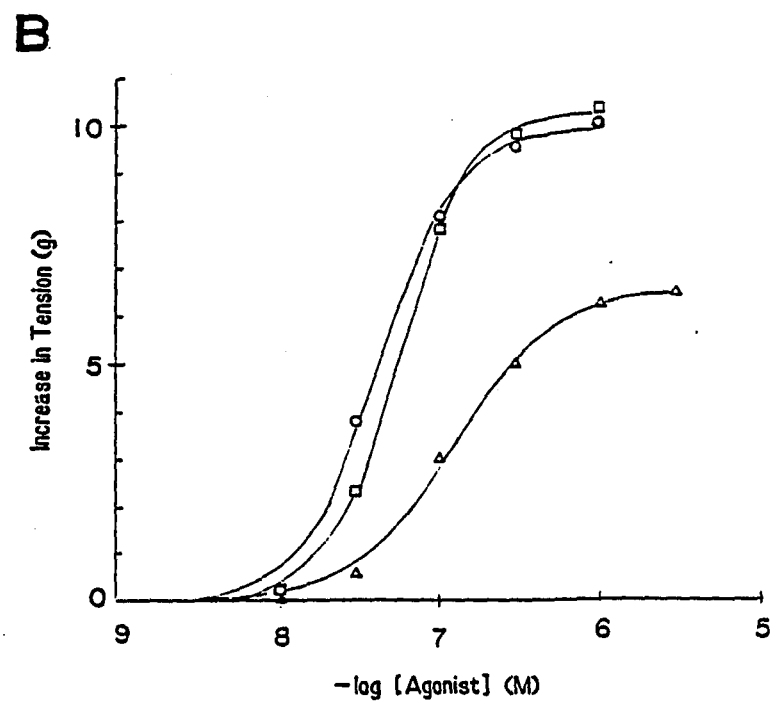

B
Increase in Tension (g)
10
5
0
9
8
7
6
5
-log [Agonist] (M)

Figure 2. The response to AMT in the absence and presence of spiperone in the isolated rabbit aorta. A. In a paired control ring, AMT a[illegible] and ◇. B. AMT assays were following order: ○, □, △ and ◇. B. AMT assays were performed in the absence (○) and presence of spiperone (nM): 2.5 (■), 10.0 (▲) and 40.0 (◆); each point represents the arithmetic mean +/- S.E.M. from three rings of tissue obtained from one rabbit. Standard error bars are shown when larger than the indicated symbols. C. A Schild plot for spiperone. The slope of the Schild plot is 1.10 (0.83-1.37), not significantly different from one (t-test; $0.2<p<0.5$). The pA_2 is 9.39 (9.27-9.51), obtained from a slope constrained to one. The numbers in parentheses are the 95% confidence limits of the slope and pA_2.

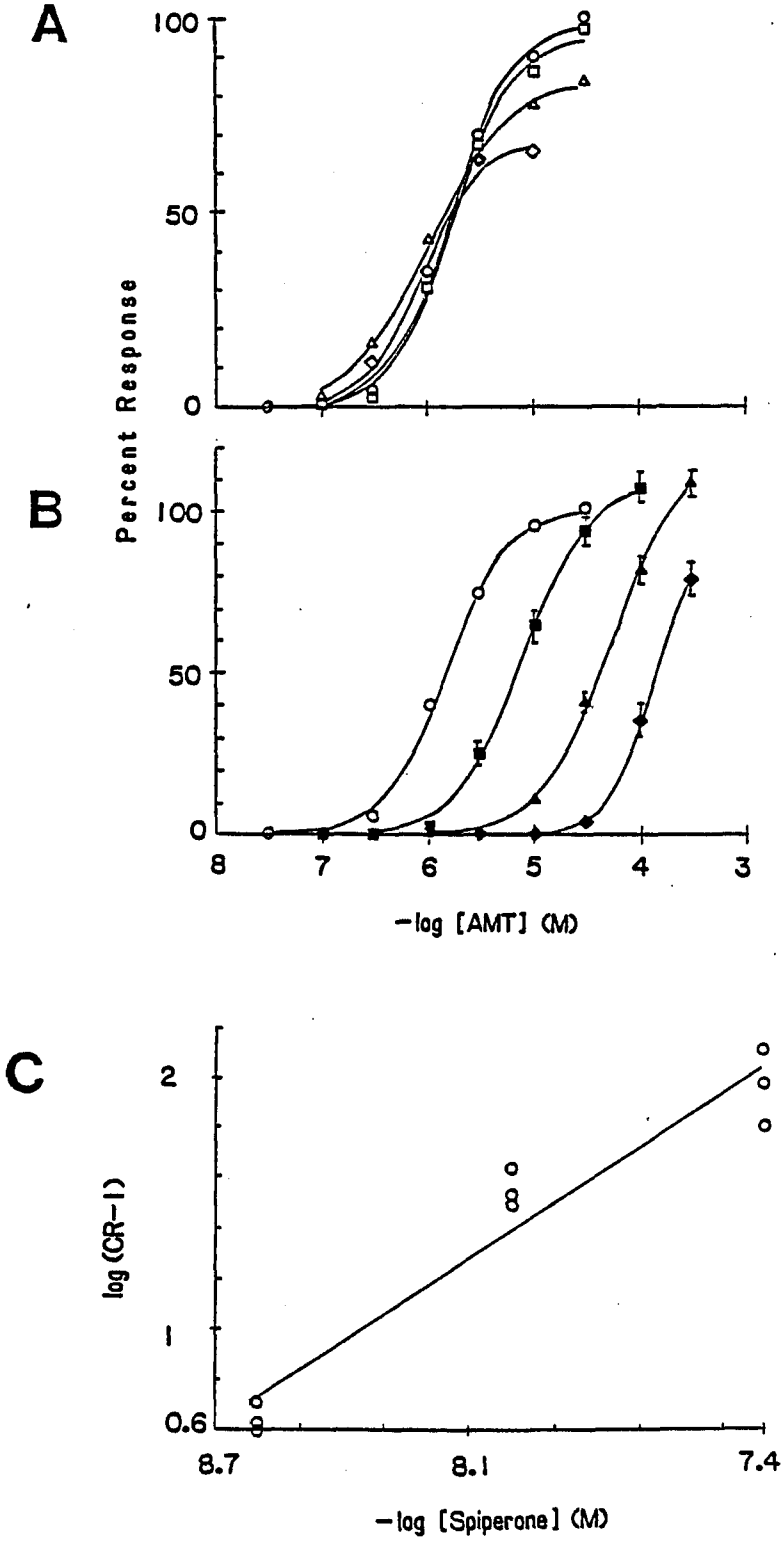

A
B
C
100
50
0
Percent Response
8
7
6
5
4
3
−log [AMT] (M)
2
1
0.6
log (CR−1)
8.7
8.1
7.4
−log [Spiperone] (M)

Figure 3. Determinations of the dissociation constants of 5-HT and 5-HDMT (BUF) at 5-HT_2 receptors in the isolated rabbit aorta with the method of fractional receptor inactivation (Method 1). A. The responses to 5-HT and 5-HDMT in the same tissue before and after the addition of dibenamine (0.1 uM for 6 min). B. Analyses of the data depicted in panel A. The dissociation constants (nM) of 5-HT (○) and 5-HDMT (□) are 190 and 165, respectively. After dibenamine, the fractions of functional receptors (q) determined with 5-HT and 5-HDMT are 0.49 and 0.40, respectively.

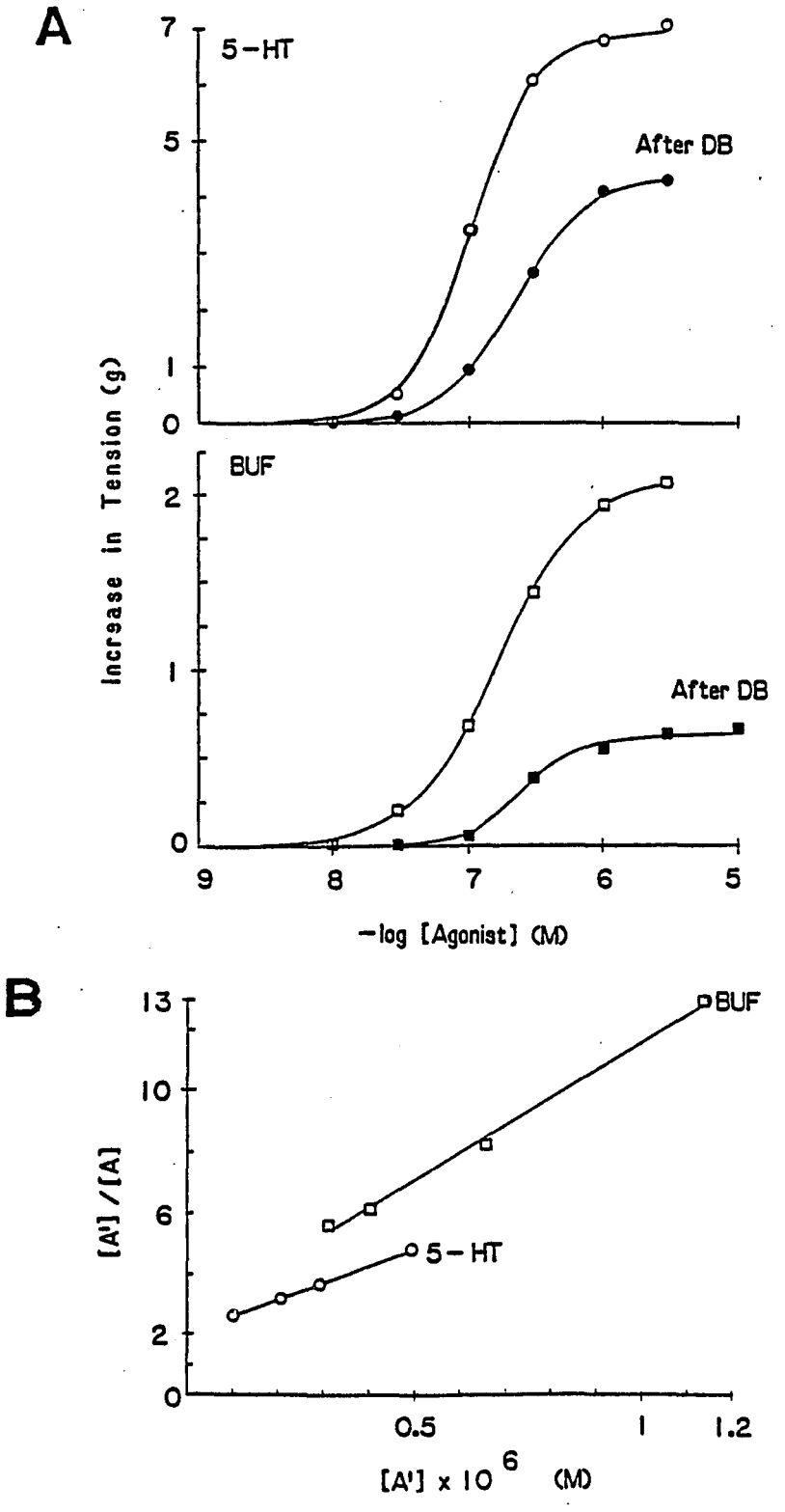

A
5-HT
After DB
BUF
After DB
Increase in Tension (g)
7
5
1
0
2
1
0
9
8
7
6
5
-log [Agonist] (M)
B
13
10
6
2
0
BUF
5-HT
[A'] / [A]
0.5
1
1.2
[A'] x 10 6 (M)

Figure 4. A comparison of the receptor occupancy-response curves of 5-HT and 5-HDMT at 5-HT_2 receptors in the isolated rabbit aorta. See Methods for details regarding the construction of this figure. The points represent the mean +/- S.E.M. of nine assays of 5-HT (○) and 5-HDMT (□) in the same tissues. The antilog of the equiactive interval between the two curves represents the relative intrinsic efficacy.

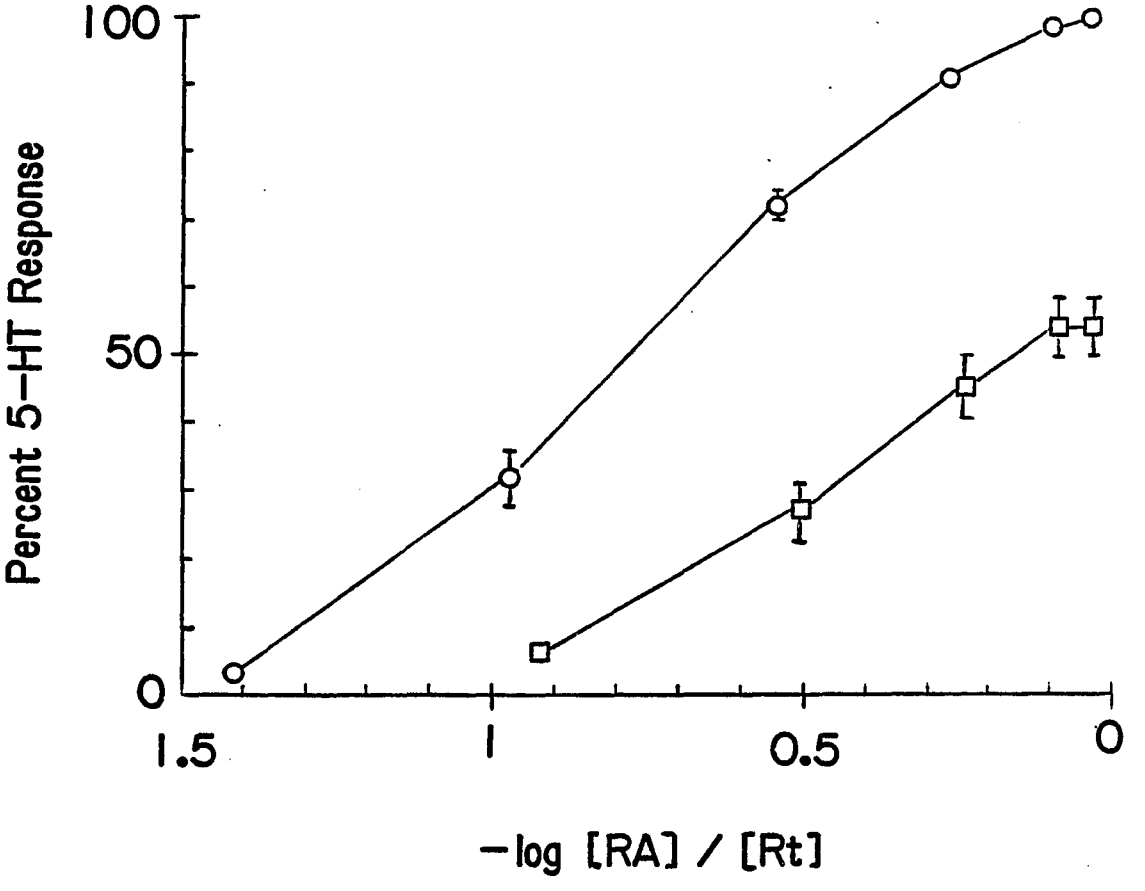

100
50
0
Percent 5-HT Response
1.5
1
0.5
0
-log [RA] / [Rt]

Figure 5. A Barlow plot (Method 2) to determine the dissociation constant (Kp) of 5-HDMT (BUF) at 5-HT_2 receptors in the isolated rabbit aorta. Concentration-response assays for 5-HT and 5-HDMT were performed consecutively on the same tissues. The data points in the Barlow plot were derived from averaged concentration-response curves (CRCs) for 5-HT and 5-HDMT; four sets of CRCs on tissues obtained from the same rabbit. The ordinate represents the molar concentrations of 5-HT that are equiactive to some concentrations of 5-HDMT. The abscissa represents the ratio of equiactive concentrations of 5-HT and 5-HDMT (BUF), i.e., [5-HT]/[5-HDMT]. Kp (equation 5) is 136 nM and the relative intrinsic efficacy value of 5-HDMT (equation 6) is 0.23.

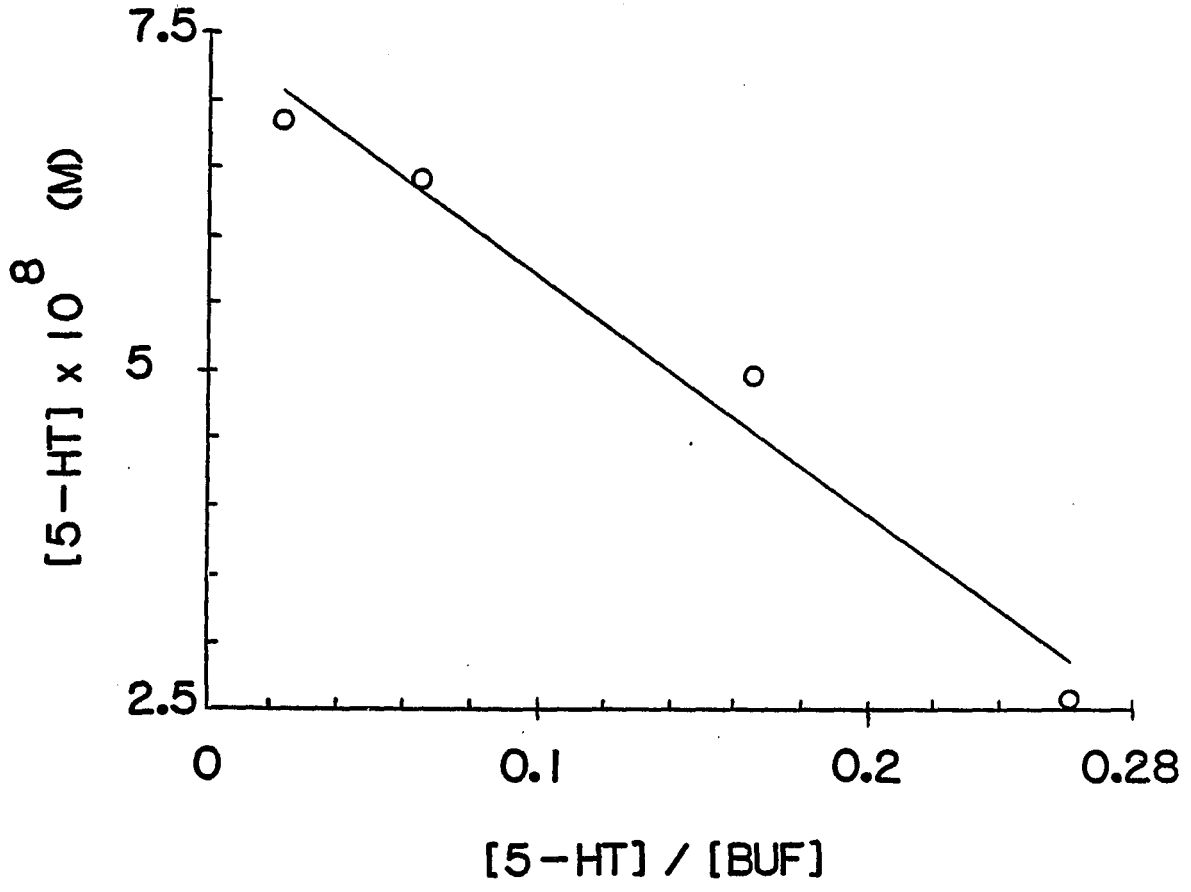
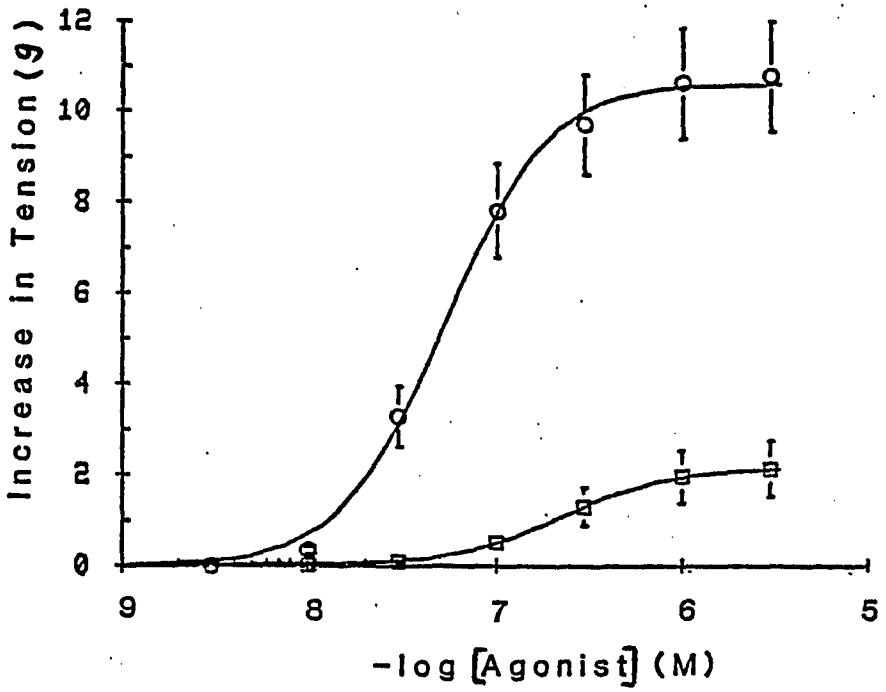

7.5
5
2.5
[5-HT] x 10^8 (M)
0
0.1
0.2
0.28
[5-HT] / [BUF]

Figure 6. The responses to 5-HT and 4-HDMT in the isolated rabbit aorta. A. Points shown are the mean +/- S.E.M. of three consecutive assays for 5-HT and 4-HDMT on the same tissues. The curves were obtained from unweighted fits of the data to equation 1. B. A Barlow plot (Method 2) to determine the dissociation constant (Kp) of 4-HDMT at 5-HT_2 receptors. The data is from one experiment. Concentration-response assays for 5-HT and 4-HDMT were performed consecutively on the same tissue as depicted in panel A. The ordinate represents the molar concentrations of 5-HT that are equiactive to some concentrations of 4-HDMT. The abscissa represents the ratio of equiactive concentrations of 5-HT and 4-HDMT, i.e., [5-HT]/[4-HDMT]. Kp (equation 5) is 186 nM and the relative intrinsic efficacy of 4-HDMT (equation 6) is 0.08.


Increase in Tension (g)
12
10
8
6
4
2
0
9
8
7
6
5
-log [Agonist] (M)

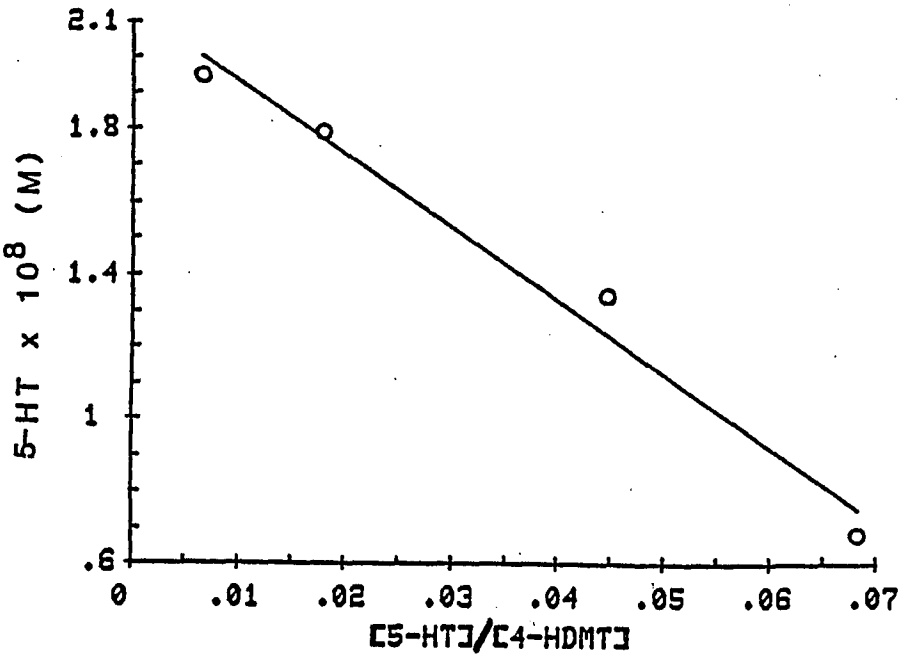
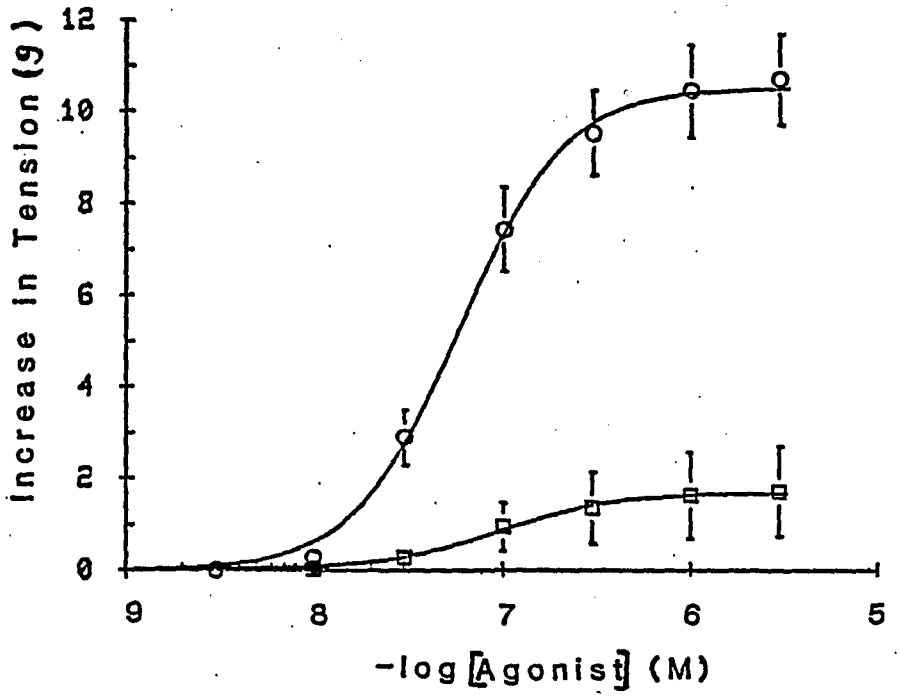

5-HT x 10^8 (M)
2.1
1.8
1.4
1
.6
0
.01
.02
.03
.04
.05
.06
.07
[5-HT]/[4-HDMT]

Figure 7. The responses to 5-HT and 4,5-MDODMT in the isolated rabbit aorta. A. Points shown are the mean +/- S.E.M. of three consecutive assays for 5-HT and 4,5-MDODMT on the same tissues. The curves were obtained from unweighted fits of the data to equation 1. B. A Barlow plot (Method 2) to determine the dissociation constant (Kp) of 4,5-MDODMT at 5-HT_2 receptors. The data is from one experiment. Concentration-response assays for 5-HT and 4,5-MDODMT were performed consecutively on the same tissue as depicted in panel A. The ordinate represents the molar concentrations of 5-HT that are equiactive to some concentrations of 4,5-MDODMT. The abscissa represents the ratio of equiactive concentrations of 5-HT and 4,5MDODMT, i.e., [5-HT]/[4,5-MDODMT]. Kp (equation 5) is 52 nM and the relative intrinsic efficacy of 4,5-MDODMT (equation 6) is 0.05.


Increase in Tension (g)
12
10
8
6
4
2
0
9
8
7
6
5
-log [Agonist] (M)

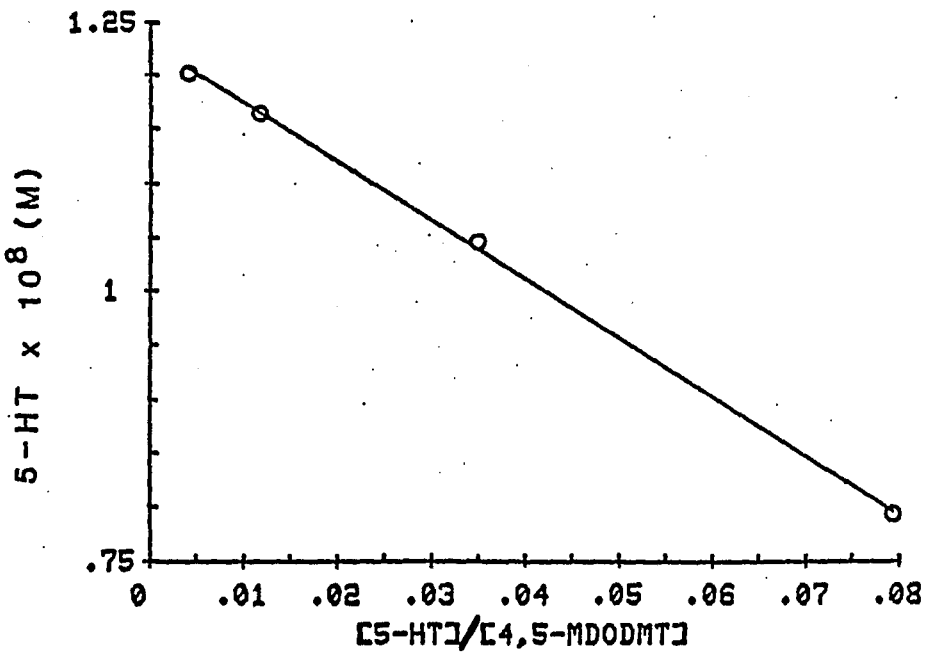
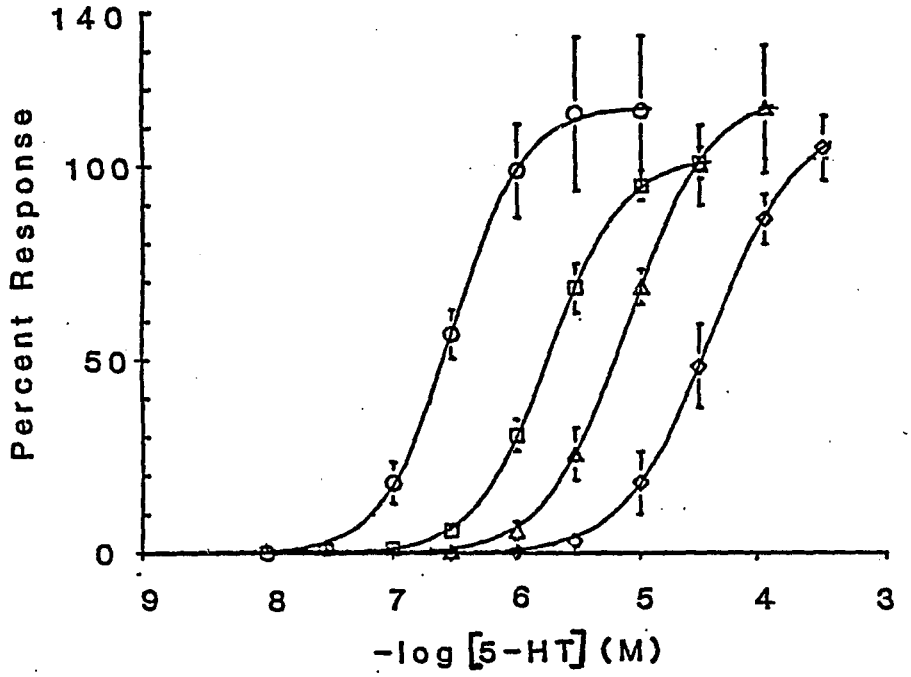

5-HT x 10^8 (M)
1.25
1
.75
0
.01
.02
.03
.04
.05
.06
.07
.08
[5-HT]/[4,5-MDODMT]

Figure 8. Antagonism of the response to 5-HT in the isolated rabbit aorta by 4-HDMT. A. 5-HT assays were performed in the absence (O) and in the presence of 4-HDMT at concentrations (uM) of 0.5 (□), 2 (Δ) and 10 (◇). Each point is the mean +/- S.E.M of three experiments. The curves were obtained from an unweighted fit of the data to equation 1. B. A Schild plot for 4-HDMT (n=3). The slope of the Schild plot is 1.02 (0.91-1.13), not significantly different from one (t-test; $0.2<p<0.5$). The pA_2 is 7.25 (7.23-7.27), obtained from a slope constrained to one. The numbers in parentheses are the 95% confidence limits of the slope and pA_2.


140
100
50
0
Percent Response
9
8
7
6
5
4
3
-log [5-HT] (M)

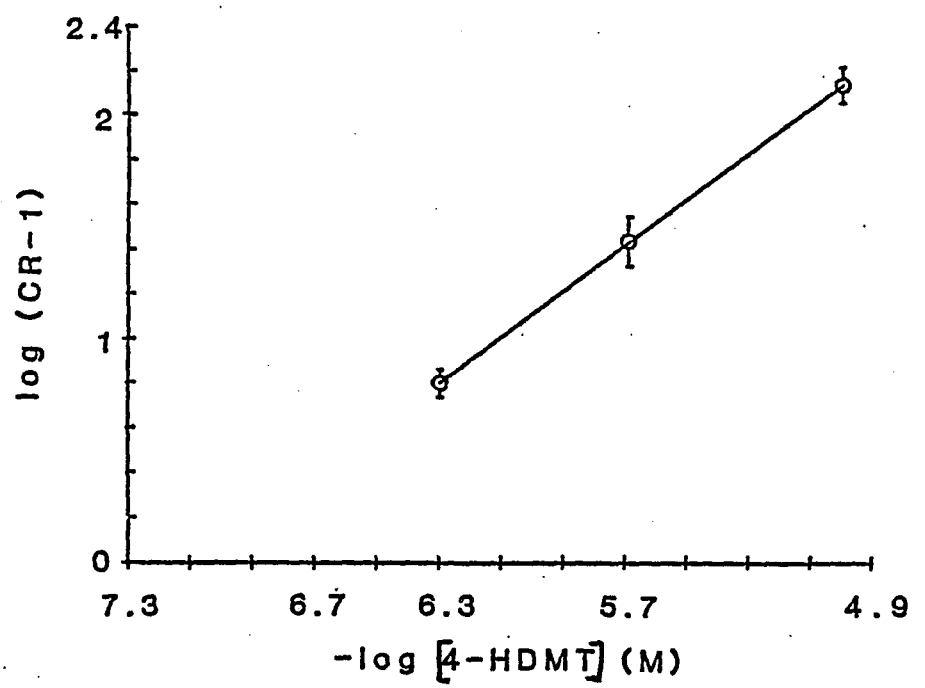
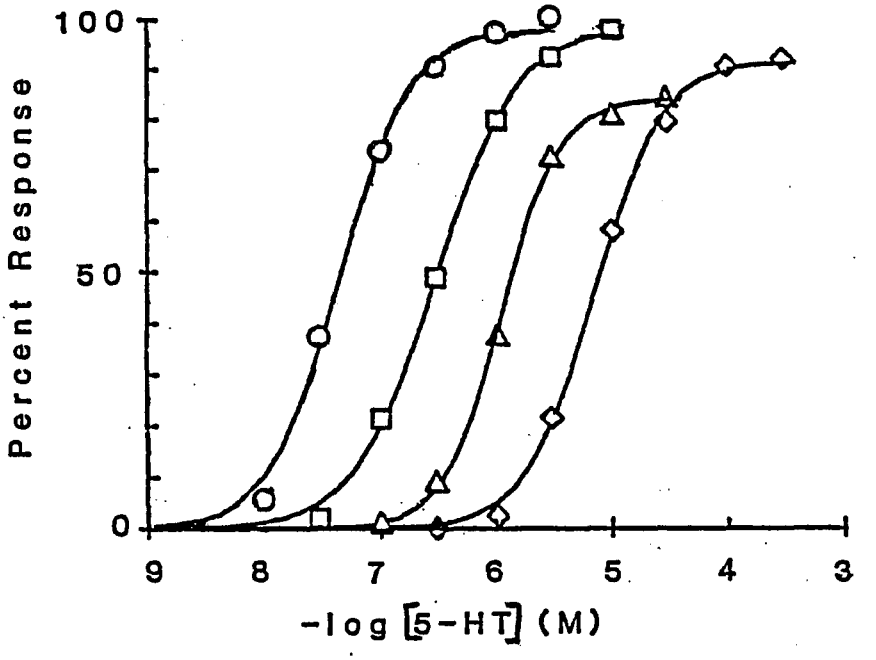

2.4
2
1
0
log (CR-1)
7.3
6.7
6.3
5.7
4.9
-log [4-HDMT] (M)

Figure 9. Antagonism of the response to 5-HT in the isolated rabbit aorta by 4,5-MDODMT. A. In a representative experiment 5-HT assays were performed in the absence (○) and in the presence of 4,5-MDODMT at concentrations (uM) of 0.1 (□), 0.4 (△) and 2 (◇). The curves were obtained from a fit of the data to equation 1. B. A Schild plot for 4,5-MODMT; data compiled from four experiments. The slope of the regression is 0.95 (0.57-1.33), not significantly different from one (t-test; $0.5<p$). The pA_2 is 7.82 (7.69-7.95), obtained from a slope constrained to one. The numbers in parentheses are the 95% confidence limits of the slope and pA_2.


100
50
0
Percent Response
9
8
7
6
5
4
3
-log [5-HT] (M)

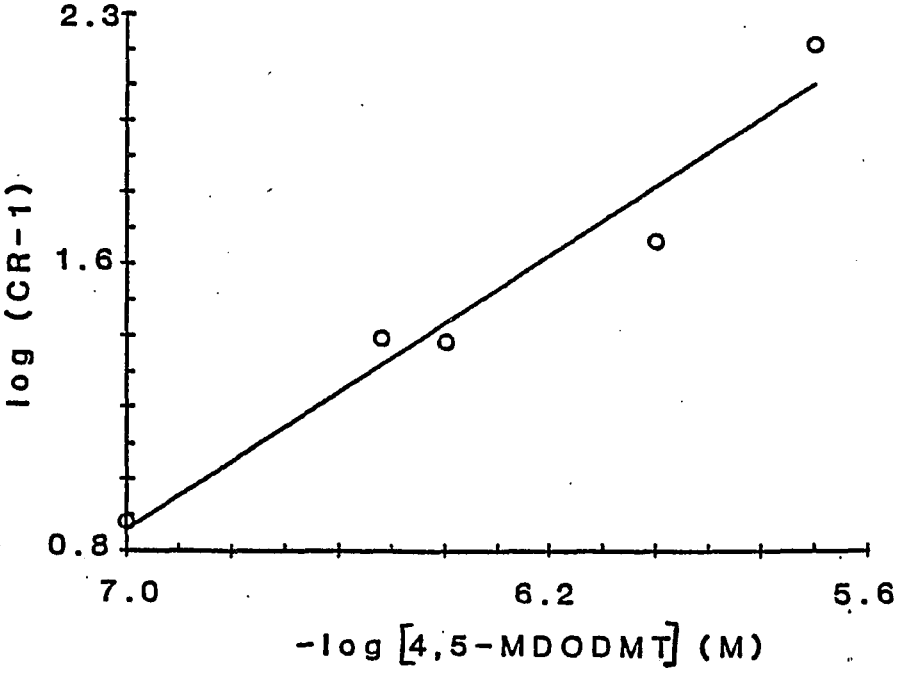

2.3
1.6
0.8
log (CR-1)
7.0
6.2
5.6
-log [4,5-MDODMT] (M)

Figure 10. Simulations of the dependence of response upon 5-HT_2 receptor occupation in the rabbit aorta for the agonists 5-HT, T and 5-HDMT (BUF). See "results" for details on the construction of this figure.

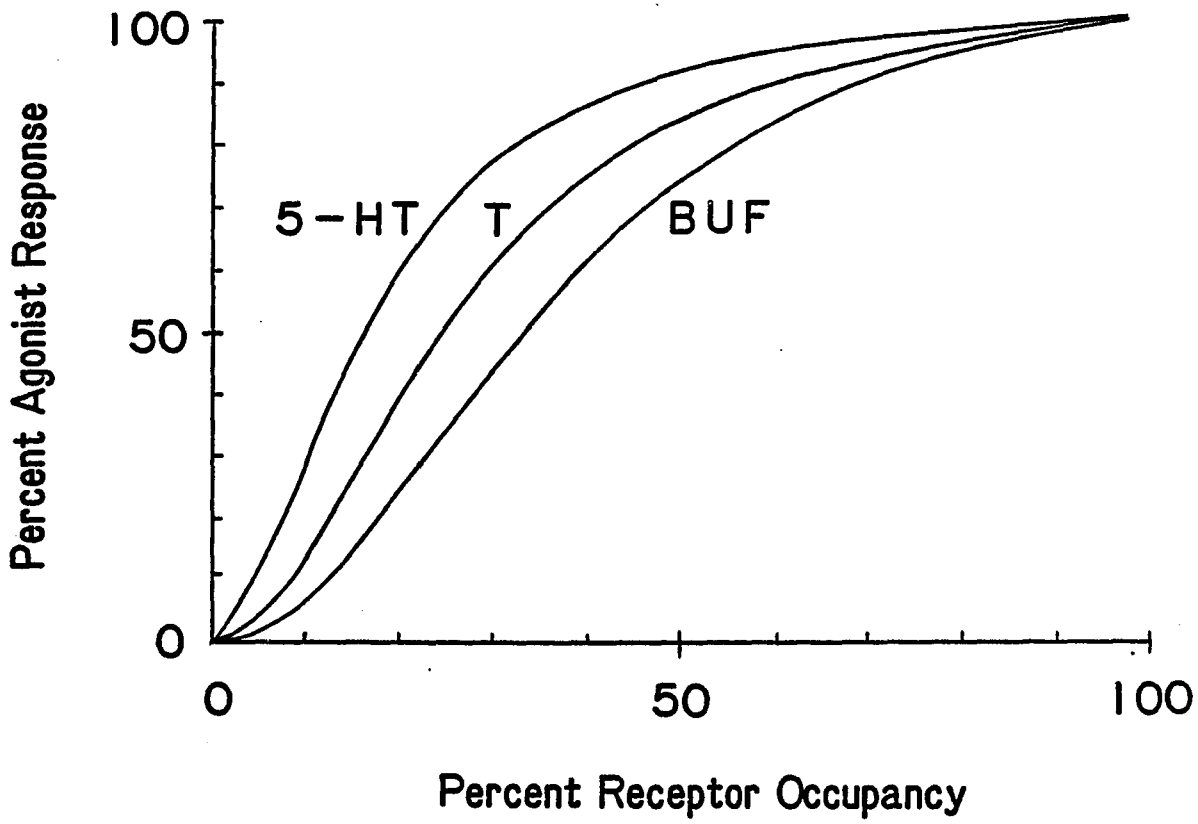

100
50
0
Percent Agonist Response
5-HT
T
BUF
0
50
100
Percent Receptor Occupancy

Figure 11. The experimental protocol for the competition kinetics method. A composite of polygraph tracings of assays performed on a single ring of the rabbit aorta in the absence of the adventitia. The agonist is 5-HT, 3 uM. The tracings represent: A. A control assay: the effect of time on the response to 5-HT. B. The response to 5-HT after the addition of 50 nM spiperone. C. The response to 5-HT after the addition of 200 nM spiperone.

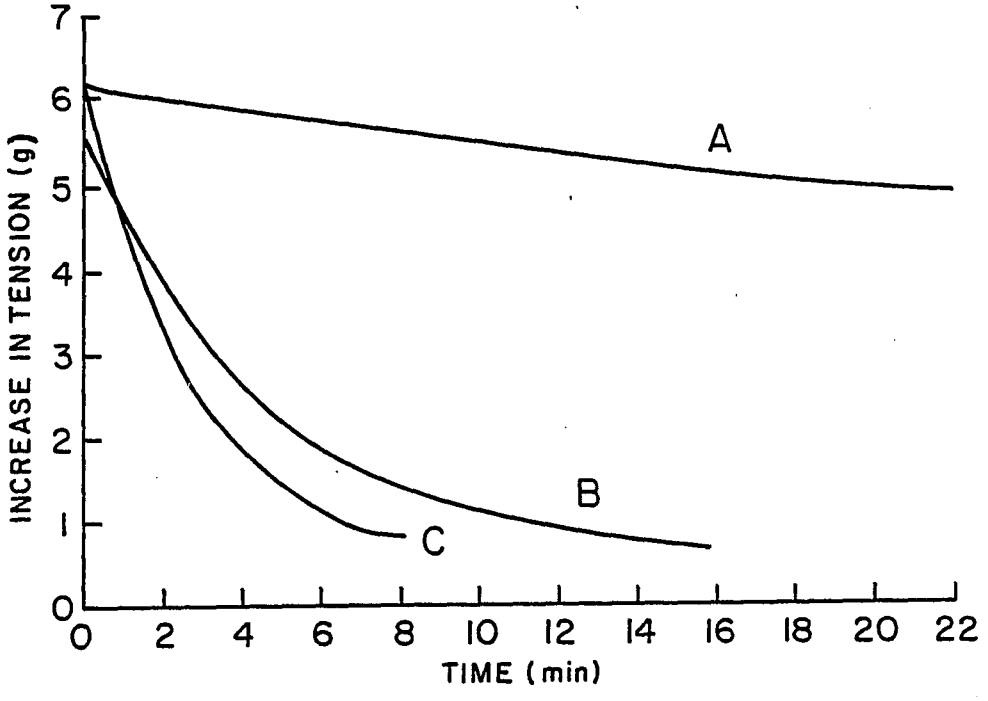

INCREASE IN TENSION (g)
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
18
20
22
TIME (min)
A
B
C

Figure 12. Kinetics of competition between 3 uM 5-HT and spiperone in the isolated rabbit aorta. The assays were performed in the absence of the adventitia. The concentrations of spiperone are 50 nM (curve A) and 200 nM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit of the model to the data. The parameter estimates +/- S.E. for this experiment are k_2 = 0.389 +/- 0.003 min^{-1}; k_3 = 131.80 +/- 2.90 $(uMmin)^{-1}$ and k_4 = 0.047 +/- 0.003 min^{-1}. The calculated dissociation constant of spiperone is 0.36 nM. Average values from several experiments are shown in Table 7.

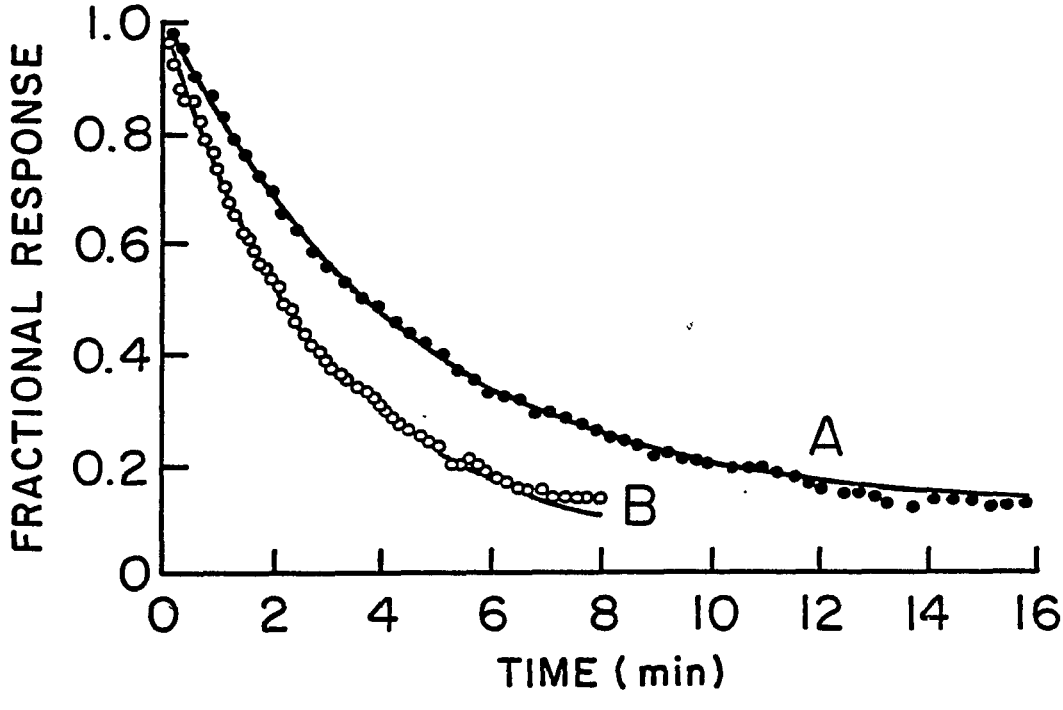

FRACTIONAL RESPONSE
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
TIME (min)
A
B

Figure 13. Kinetics of competition between 3 uM 5-HT and 5-MeOG in the isolated rabbit aorta. The assays were performed in the absence of the adventitia. The concentrations of 5-MeOG are 10 uM (curve A) and 3 uM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit of the model to the data. The parameter estimates +/- S.E. for this expriment are k_2 = 0.36 +/- 0.01 min^{-1}; k_3 = 0.67 +/- 0.02 $(uMmin)^{-1}$ and k_4 = 0.19 +/- 0.01 min^{-1}. The calculated dissociation constant of 5-MeOG is 0.28 uM. Average values from several experiments are shown in Table 7.

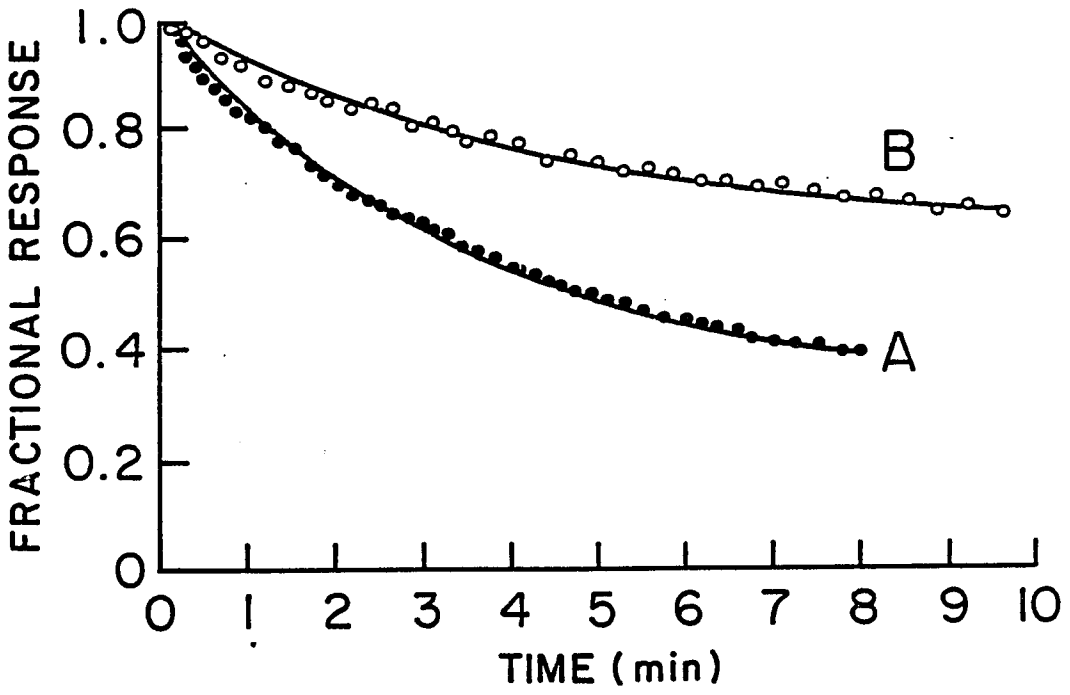

FRACTIONAL RESPONSE
1.0
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
8
9
10
TIME (min)
B
A

Figure 14. Kinetics of competition between 3 uM 5-HT and spiperone in the isolated rabbit aorta. The assays were performed in the presence of the adventitia. The concentrations of spiperone are 50 nM (curve A) and 15 nM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit of the model to the data. The parameter estimates +/- S.E. for this experiment are k_2 = 0.326 +/- 0.006 min^{-1}; k_3 = 144.40 +/- 2.55 $(uMmin)^{-1}$ and k_4 = 0.036 +/- 0.001 min^{-1}. The calculated dissociation constant of spiperone is 0.25 nM. Average values from several experiments are shown in Table 8.

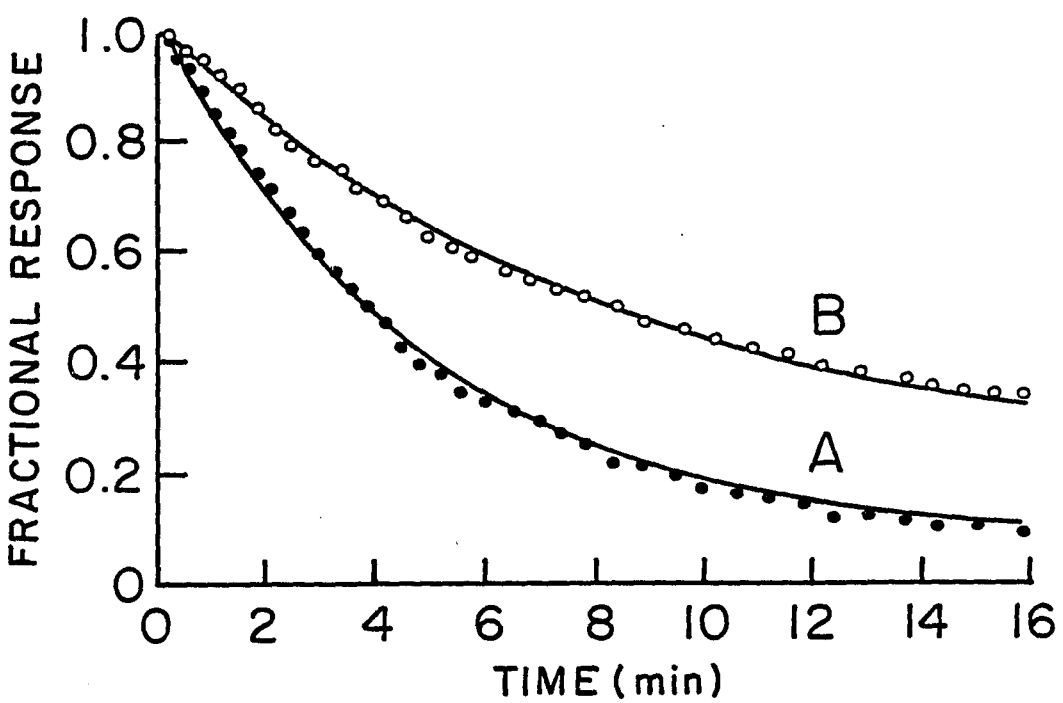

FRACTIONAL RESPONSE
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
TIME (min)
B
A

Figure 15. Kinetics of competition between 3 uM 5-HT and 5-MeOG in the isolated rabbit aorta. The assays were performed in the presence of the adventitia. The concentrations of 5-MeOG are 10 uM (curve A) and 30 uM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit of the model to the data. The parameter estimates +/- S.E. for this experiment are k_2 = 0.60 +/- 0.02 min^{-1}; k_3 = 0.25 +/- 0.01 $(uMmin)^{-1}$ and k_4 = 0.09 +/- 0.01 min^{-1}. The calculated dissociation constant of 5-MeOG is 0.36 uM. Average values from several experiments are shown in Table 8.

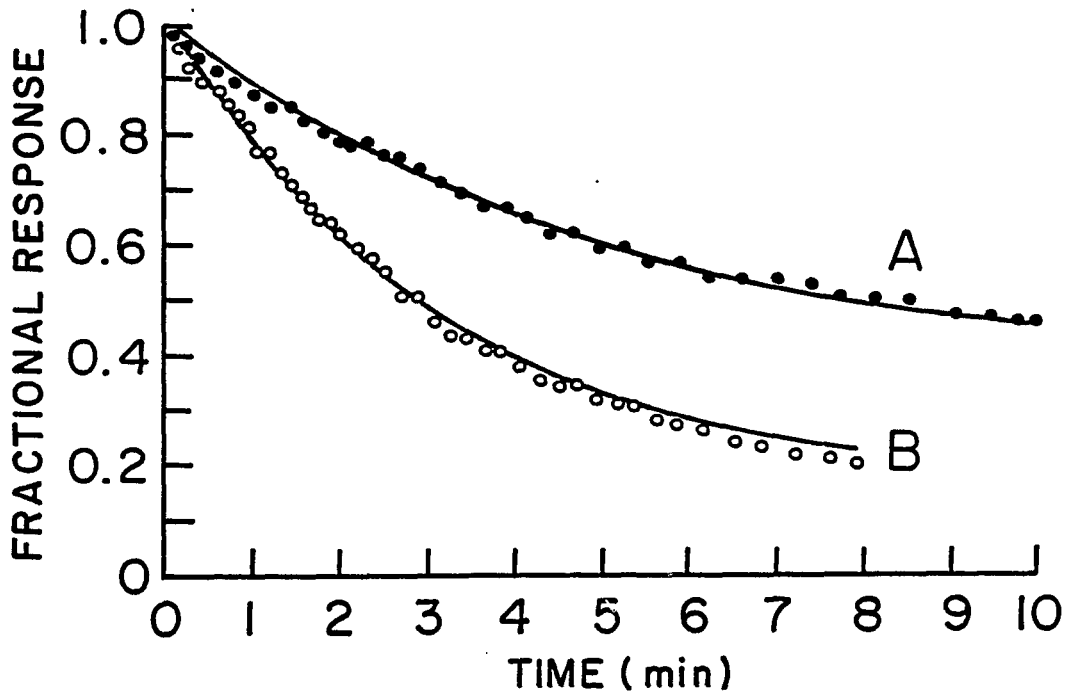

FRACTIONAL RESPONSE
1.0
0.8
0.6
0.4
0.2
0
A
B
0 1 2 3 4 5 6 7 8 9 10
TIME (min)

Figure 16. The effect of nonsurmountable antagonists on the response to 5-HT in the isolated rabbit aorta. Three assays were performed in each tissue. Points shown are the mean values from three experiments and the vertical line shows the standard error. The curves were obtained from an unweighted fit of the data to equation 1. A. Concentration-response curves in the absence and presence of LSD or LSD and spiperone.

Curve	symbol	[antag.](nM)	EC50(nM)	Emax(%)	slope
1	○	--	82	100	1.50
2	△	6 nM LSD	3110	25	0.90
3	□	6 nM LSD + 25 nM spip	6200	73	1.30

B. Concentration-response curves in the absence and presence of IOL or IOL and spiperone.

Curve	symbol	[antag.](nM)	EC50(nM)	Emax(%)	slope
1	○	---	73	100	1.73
2	△	12.5 nM IOL	2230	62	1.30
3	□	12.5 nM IOL + 40 nM spip	8600	94	1.52

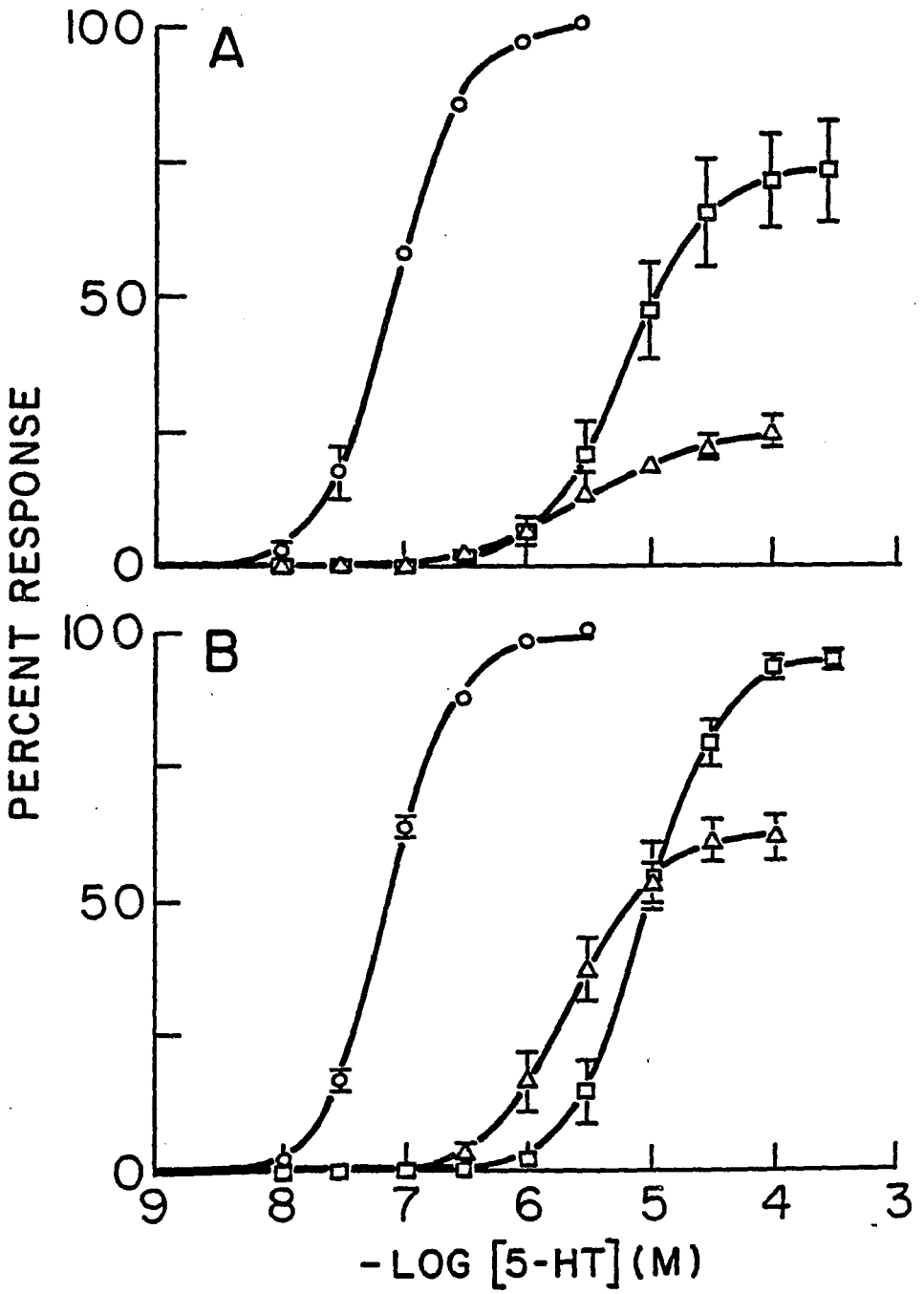

100
A
50
0
PERCENT RESPONSE
100
B
50
0
9
8
7
6
5
4
3
-LOG [5-HT] (M)

Figure 17. The response to 5-HT in the isolated rabbit aorta in the absence and presence of IOL. Three to four assays were performed in each tissue. Vertical lines show the standard error of the mean. The curves were obtained from an unweighted fit of the data to equation 1 with the following results:

Curve	n	symbol	[IOL](nM)	EC50(nM)	Emax(%)	slope
1	3	○	--	70	100	1.44
2	2	□	2.5	260	73	1.51
3	3	△	12.5	1420	71	1.27
4	3	X	50	5090	67	1.13

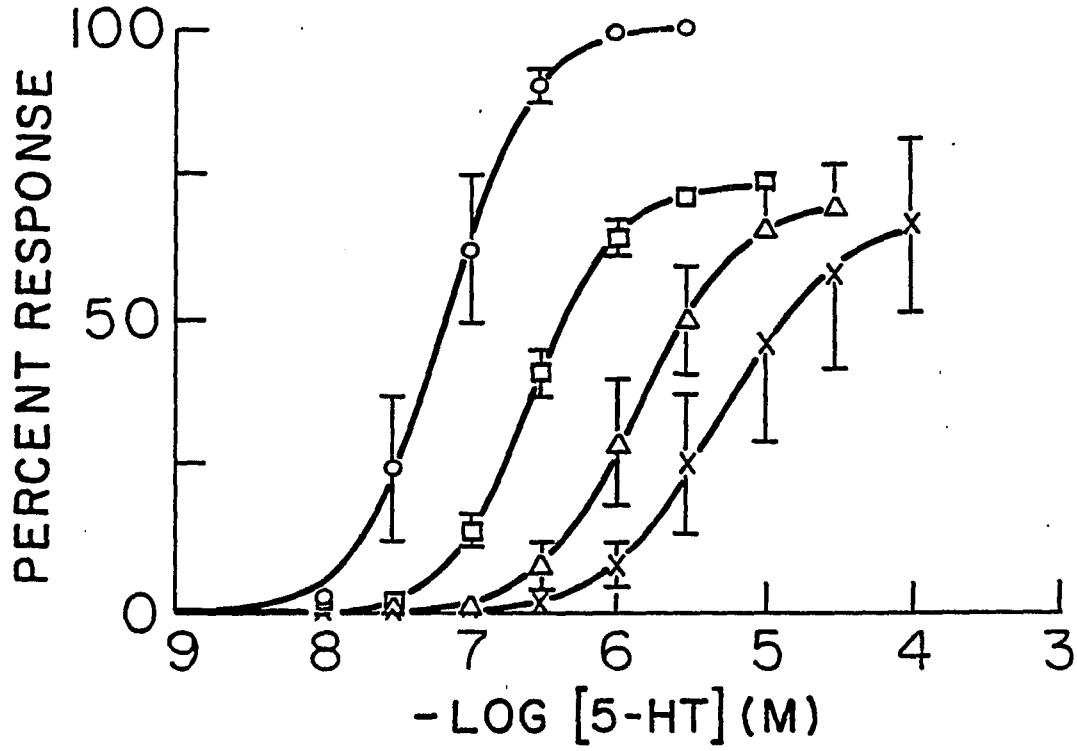

100
50
0
PERCENT RESPONSE
9
8
7
6
5
4
3
-LOG [5-HT] (M)

Figure 18. LSD-induced decreases in the maximal response to 5-HT. The concentration-response curve of LSD in the isolated rabbit aorta (○,n=4). A. Antagonism of the response to LSD by 10 nM ketanserin (□,n=2). The K_B of ketanserin, calculated from equieffective concentration ratios, is 1.17 +/- 0.13 nM. B. Antagonism of the response to LSD by 6 uM 5-MeOG (△,n=2). The K_B of 5-MeOG is 0.58 +/- 0.08 uM. The vertical lines show the standard error of the mean.

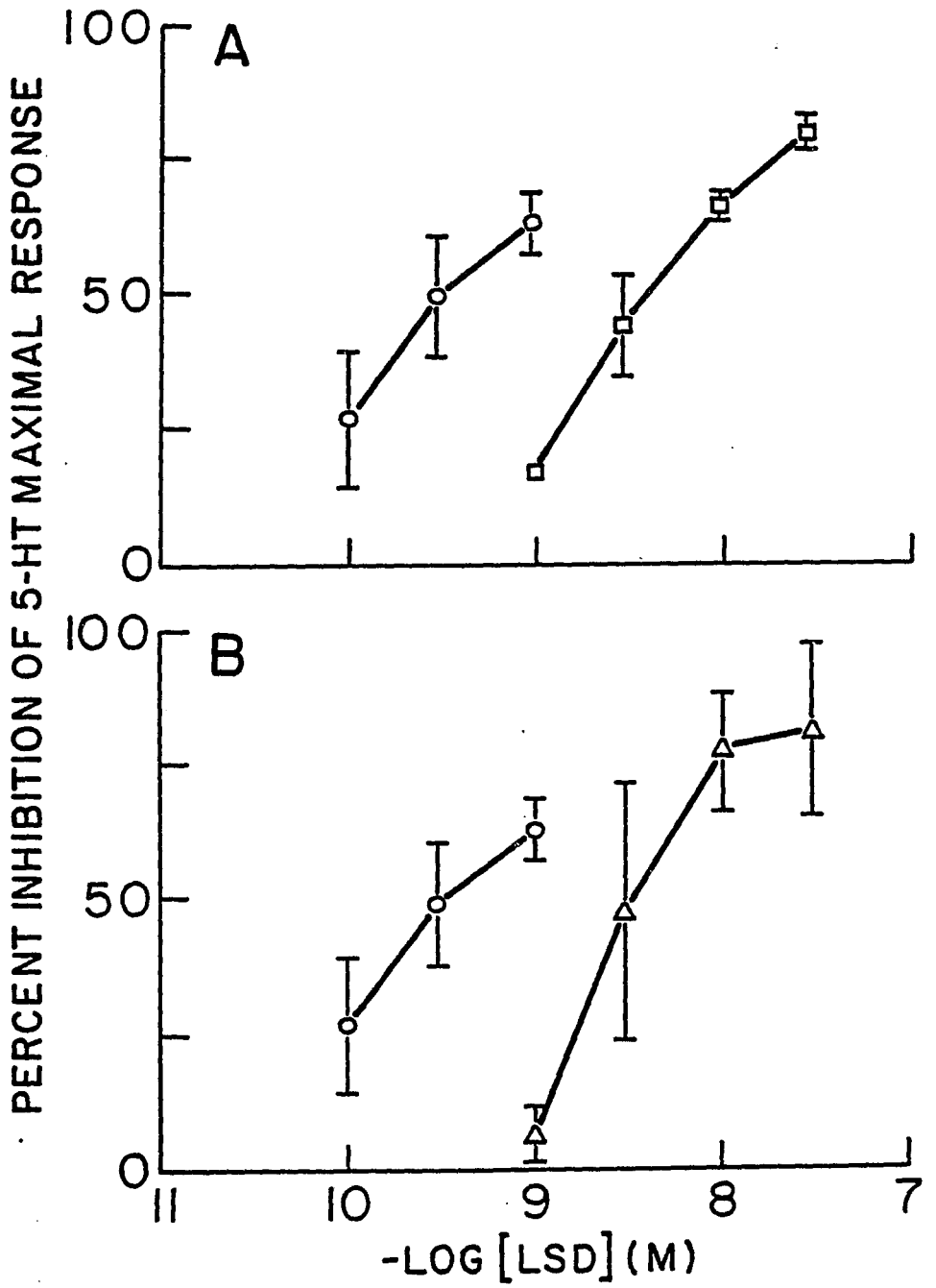

A
B
100
50
0
PERCENT INHIBITION OF 5-HT MAXIMAL RESPONSE
11
10
9
8
7
-LOG [LSD] (M)

Figure 19. Kinetics of competition between 3 uM 5-HT and methysergide in the isolated rabbit aorta. The assays were performed in the absence of the adventitia. The concentrations of methysergide are 100 (curve A) and 400 nM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit of the model to the data. The parameter estimates +/- S.E. are k_2 = 0.60 +/- 0.02 min^{-1}; k_3 = 25.7 +/- 0.8 $(uMmin)^{-1}$ and k_4 = 0.070 +/- 0.004 min^{-1}. The calculated dissociation constant of methysergide, i.e. k_4/k_3, is 2.6 nM. Average values from several experiments are shown in table 7.

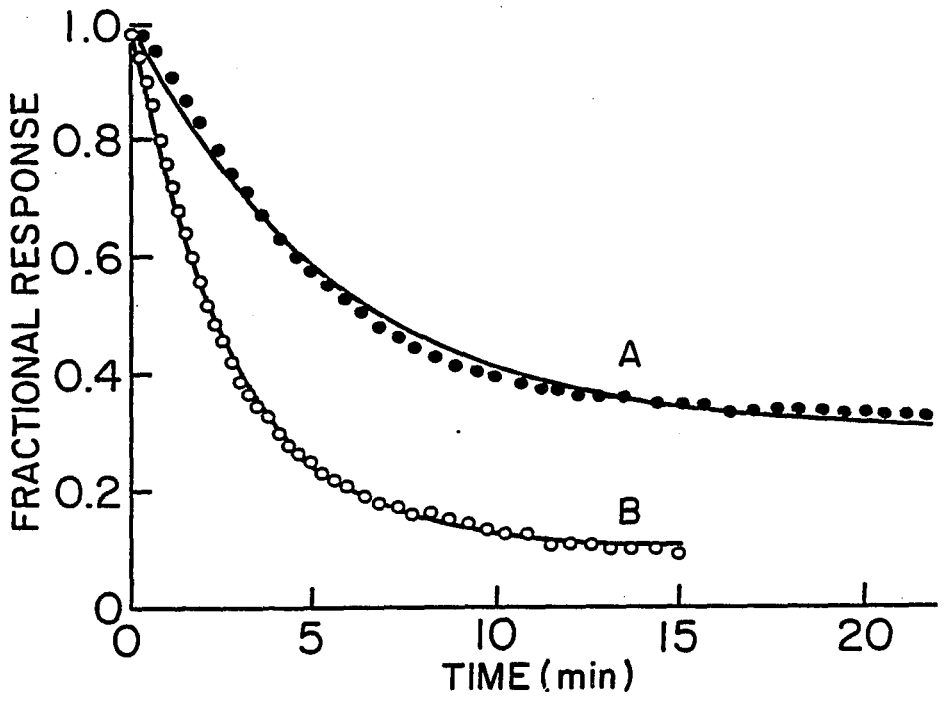

1.0
0.8
0.6
0.4
0.2
0
FRACTIONAL RESPONSE
A
B
0
5
10
15
20
TIME (min)

Figure 20. Kinetics of competition between 3 uM 5-HT and LSD. The assays were performed in the absence of adventitia. The concentrations of LSD are 50 nM (curve A) and 400 nM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit of the model to the data. Parameter estimates +/- S.E are k_2 = 0.35 +/- 0.01 min^{-1}; k_3 = 32.5 +/- 1.3 $(uMmin)^{-1}$ and k_4 = -0.025 +/- 0.003 min^{-1}. This experiment was performed three times with similar results.

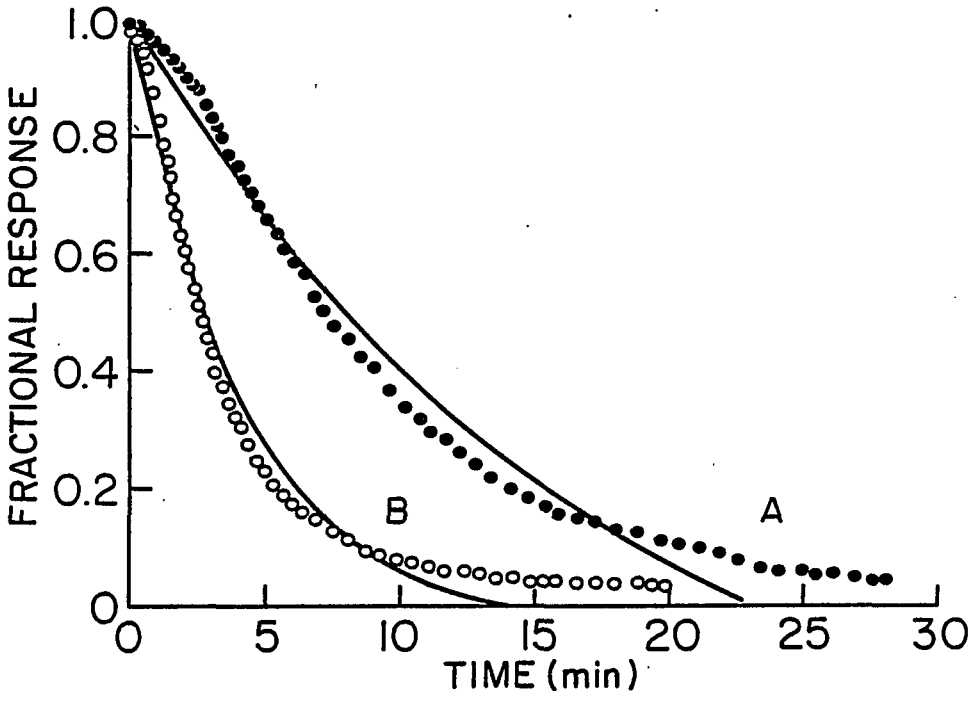

FRACTIONAL RESPONSE
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
TIME (min)
A
B

Figure 21. Kinetics of competition between 3 uM 5-HT and IOL. The assays were performed in the absence of adventitia. The concentrations of IOL are 50 nM (curve A) and 400 nM (curve B). The ordinate represents the fractional response which is related to $[AR]/[AR]_0$ (see Methods). The curves show the fit the model to the data. Parameter estimates +/- S.E. are k_2 = 0.156 +/- 0.005 min^{-1}; k_3 = 18.45 +/- 0.90 $(uMmin)^{-1}$ and k_4 = -0.05 min^{-1}. This experiment was performed three times with similar results.

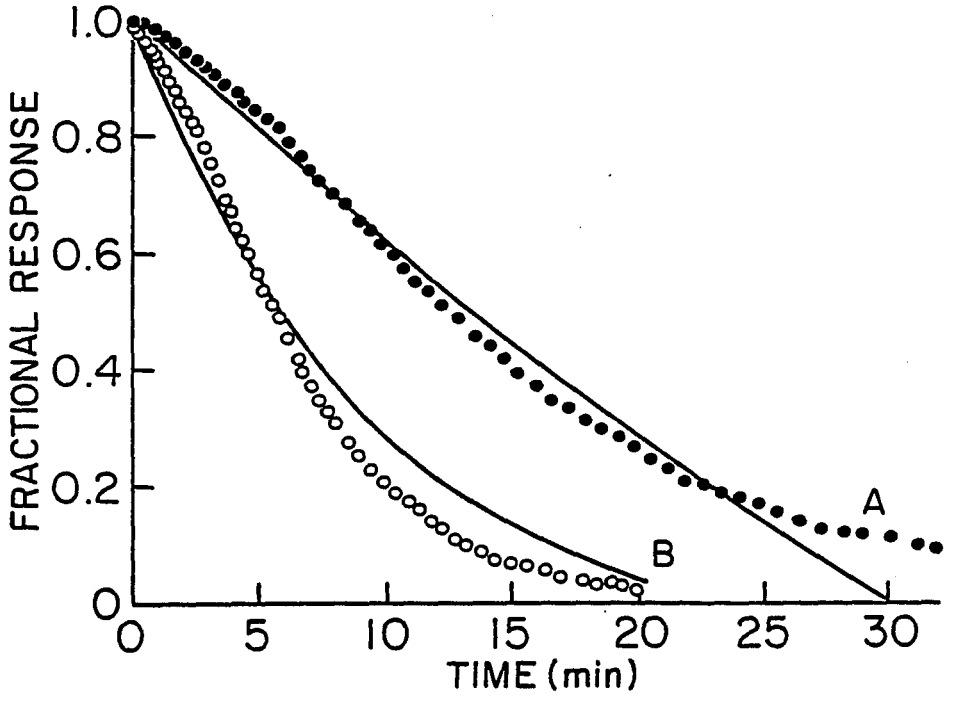
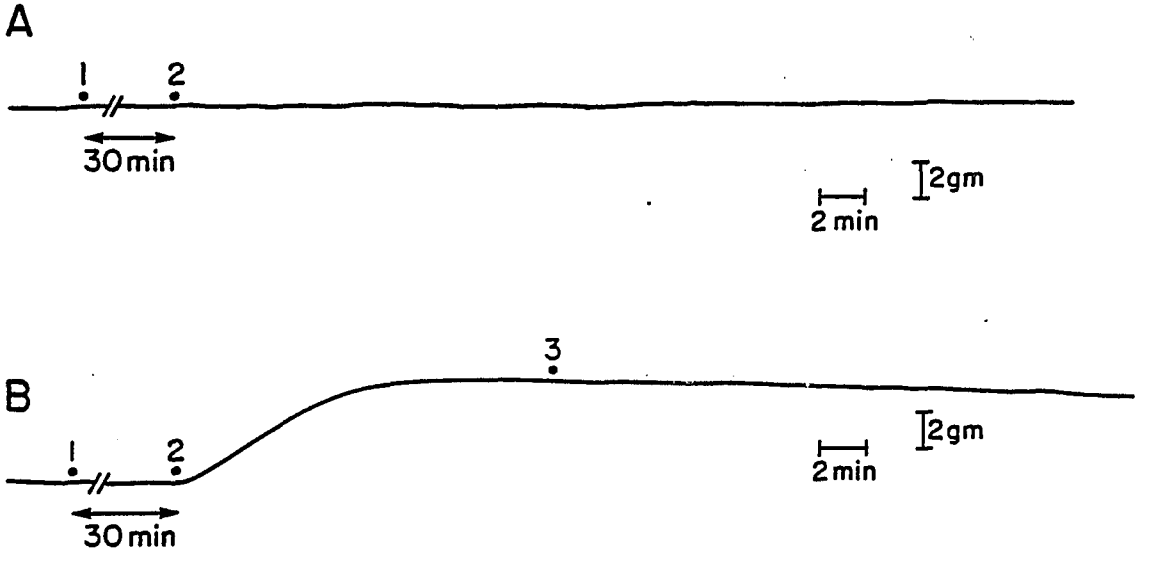

FRACTIONAL RESPONSE
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
TIME (min)
A
B

Figure 22. Tracings of the contractile response to LSD in the isolated rabbit aorta in the presence of 300 nM Bay K 8644. A. The effect of 20 nM LSD 30 min after the addition of 200 nM spiperone and 300 nM Bay K 8644. Bay K 8644 and spiperone were added at 1 and LSD was added at 2. This experiment was perfomed three times with similar results. B. A competition kinetics assay with 20 nM LSD and 200 nM spiperone. Bay K 8644 was added at 1; LSD was added at 2 and spiperone was added at 3. This experiment was performed three times with similar results.


A
1
2
30 min
2 min
2gm
B
3
1
2
2 min
2gm
30 min

Discussion

As reviewed in the introduction, there are discrepancies in the pharmacological characteristics of 5-HT_2 receptors which comprise differences in: i) the apparent nature of antagonist action, e.g., surmountable versus nonsurmountable antagonism, and ii) the values of the antagonist dissociation constants. These discrepancies are observed with conventional antagonists that are chemically unrelated to 5-HT or are structurally larger than 5-HT. Nonsurmountable antagonism is not unique to studies of 5-HT_2 receptors; it also occurs in studies of other 5-HT receptor subtypes, e.g., 5-HT receptors in the rat stomach fundus (Clineschmidt et al., 1985) and 5-HT_3 receptors (Ireland and Tyers, 1987), and other hormone receptors such as muscarinic (Paton and Rang, 1966) and alpha$_1$-adrenergic (Alosachie and Godfraind, 1986) receptors. The study of nonsurmountable antagonism is important because they may it may reveal information about the physiological system or about drug interactions with receptors (see Introduction). In addition, it is possible that these antagonist complications may impede the proper classification of 5-HT receptors and therefore an alternative approach may be required. A pharmacological alternative to the conventional 5-HT antagonists are agonists or antagonists that are structurally similar to the natural agonist.

This investigation extended the pharmacological characterization of 5-HT_2 receptors in three ways. First, attempts were made to identify some of the structural requirements for recognition at this receptor and activation of this receptor by simple tryptamine analogs. This information could be applied to the development of selective agonists and competitive antagonists which could contribute to the classification of 5-HT receptors. Second, the kinetic characteristics of some agonist and competitive antagonist interactions with 5-HT_2 receptors were studied. These results complemented the information obtained from the traditional steady-state methods. Third, the actions of LSD and IOL were studied in an attempt to understand their mechanism of action as nonsurmountable antagonists of the response to 5-HT in the rabbit aorta. These results may reveal how some other drugs act as nonsurmountable antagonists at this and other receptors.

I. Studies with tryptamine analogs.

As summarized in table 4 all of the tryptamine analogs tested were intrinsically active. Spiperone quantitatively identified the receptor activated by most of the agonists. The pK_B values of spiperone (table 5) indicated that the contractile response to these agonists was mediated by 5-HT_2 receptors. It should

be noted that spiperone is not a selective 5-HT_2 antagonist. Spiperone binds to $alpha_1$-adrenergic, dopamine-D_2 (Leysen et al., 1981) and 5-HT_{1A} (Pedigo et al., 1981) binding sites. However, in this study spiperone was operationally selective due to the inactivation of alpha-adrenergic receptors with BHC and the apparent absence of dopamine receptors (contractile and relaxation responses to dopamine appear to be mediated by $alpha_1$- and beta-adrenergic receptors, respectively; Besse and Furchgott, 1976) in this tissue. It is unlikey that 5-HT_{1A} receptors contribute to the contractile effect of 5-HT because i) the high affinity (K_D about 4 nM) 5-HT_{1A} ligand 8-hydroxy-2-(di-n-propylamino)-tetralin (Middlemiss and Fozard, 1983; Gozlan et al., 1983) is a partial agonist with low affinity for 5-HT_2 receptors in the bovine coronary artery (Frenken and Kaumann, 1984) and the rabbit aorta (EC50 = 20 uM; not included in results section); ii) the orders of potency of 5-HT and 5-carboxamidotryptamine (5-CT) for 5-HT_{1A} and 5-HT_2 receptors are 5-CT>5-HT (Shenker et al., 1985; De Vivo and Maayani, 1986) and 5-HT>5-CT (Feniuk et al., 1981), respectively.

4-HDMT and 4,5-MDODMT, two of the weaker agonists (table 4), were competitve antagonists of the response to 5-HT. Their EC50 values (table 4) and agonist and antagonist dissociation constants (table 6) were roughly similar, indicating that the response to these drugs was

mediated by 5-HT_2 receptors. Although 6-HT and 7-HT showed activity in this preparation it was not possible to determine the identity of the receptor(s) that mediated the response.

As shown in tables 4 and 6 most of the agonist dissociation constants are greater than or equal to their EC50 values. The dissociation constants of 5-HT, 5-MeOT, 4-HT and 4,5-MDOT are about three to four times greater than their EC50 values, consistent with the possibility that these drugs are full agonists with a small receptor reserve. By definition, partial agonists do not possess a receptor reserve (Stephenson, 1956) and so it is often assumed that the relation between occupancy and their response is linear. Therefore it is tempting when inspecting the CRCs of partial agonists to equate the EC50 value with the dissociation constant. However, the occupancy-response relation for a partial agonist is not always linear (Ruffolo, 1982; Black and Leff, 1983; Kenakin, 1984) as shown with 5-HDMT in figure 10. The three curves in figure 10 compare agonist efficiencies in transducing receptor occupation into response, which is equivalent to relative efficacy (Black and Leff, 1983). If it is assumed that agonist occupation is described by equation 4 then these curves may reflect the nature of coupling between occupancy and response. The sigmoidal nature of these curves appears to occur when the slope index of the CRC of the agonist is greater than one (Black

and Leff, 1983; Kenakin, 1984). In these experiments the slope index of the CRC of 5-HT was about 1.6, consistent with previous results (Maayani et al., 1984). Analyses of the CRCs of 5-HT (with equation 1) from previous studies in the rabbit aorta (Apperley et al., 1976; Purdy et al., 1981; Stollak and Furchgott 1983) yielded slope indices of 1.25, 1.6 and 1.6, respectively. In addition the slope indices of the CRCs of all the agonists tested in this investigaton were about 1.5. Depending upon the efficacy of the partial agonist and the slope index of the CRC, the EC50 may be a poor estimate of the drug dissociation constant (Black and Leff, 1983; Black et al., 1985). For example, one of the predictions of the operational model of pharmacological agonism is that for steep CRCs the EC50 value of a weak agonist may be an overestimate of the drug dissociation constant (Black et al., 1985). This prediction is borne out by the results obtained with 4-HDMT and 4,5-MDODMT and demonstrates the need to determine the dissociation constants of partial agonists by direst methods as described in this work.

For the agonist dissociation constant to be a useful parameter in receptor classification this value should transcend species differences and anatomical location. Few studies have been conducted in which the dissociation constants of tryptamine analogs were determined in preparations that possess 5-HT_2 receptors. The dissociation constants of 5-HT and T were previously

determined in rabbit aorta (Stollak, 1980), by the procedure of fractional receptor inactivation (Method 1), and were found to be 320 and 4280 nM, respectively. Black et al. (1985) determined the dissociation constant of 5-HT in the rabbit aorta with Method 1 and obtained a value of 150 nM. In this study the dissociation constant of 5-HT (250 nM, Method 1) agreed with values obtained by others. 5-HT-induced contractions of the rat aorta and the rat external jugular vein were reported to be mediated by 5-HT_2 receptors (Cohen et al., 1981; Cohen et al., 1983). The dissociation constants of 5-HT in the rat aorta and the rat jugular vein (Cohen et al., 1981) were 6600 and 830 nM, respectively. If these preparations possess 5-HT_2 receptors then these values should be similar to each other and to the values obtained in the rabbit aorta. Clearly, the dissociation constant of 5-HT measured in the rat aorta does not agree with those values measured in either the rat jugular vein or the rabbit aorta. The values obtained in the rabbit aorta (this study; Stollak, 1980; Black et al., 1985) and the rat jugular vein (Cohen et al., 1981) are more similar but they are probably significantly different. It seems likely that the discrepancies in the dissociation constants of 5-HT between the rat and rabbit tissue preparations are due to differences in the experimental conditions used to test the response to 5-HT. Recently, evidence has been provided which indicates that the muscle

cells of the rat aorta have a large capacity of cocaine-sensitive extraneuronal uptake of 5-HT (Fukada et al., 1986; Cory et al., 1986a). This uptake system may reduce the concentration of 5-HT in the tissue bath and thereby displace the CRC of 5-HT significantly to the right of where it should be. Consequently, the agonist dissociation constant would be overestimated. Furthermore, Cohen et al. (1981) did not attempt to inhibit the enzyme monoamine oxidase (MAO) or block alpha-adenergic receptors in either the rat aorta or the rat jugular vein. Leff et al. (1986) determined the dissociation constants and relative intrinsic efficacy (RIE) values of four tryptamine analogs in the rabbit aorta and the rat jugular vein. In their experiments both tissues were exposed to pargyline, an inhibitor of MAO, and BHC, an irreversible alpha-adrenergic antagonist. Leff et al. (1986) showed that the dissociation constants and RIE values of the four agonists were virtually the same in both preparations. In their studies the dissociation constants of 5-HT and N,N-dimethyltryptamie (DMT) were 130 and 460 nM, respectively, and were similar to the values determined for 5-HT and DMT in table 6. The RIE values of DMT in both the rabbit aorta and rat jugular vein was 0.36 (Leff et al., 1986) as compared to the value of 0.16 obtained in this study (table 6). The careful work of Leff et al. (1986) supports the concept that agonist dissociation constants and RIE values can be used

to classify receptors.

For agonists with relative intrinsic activities (RIA) greater than 0.9 the dissociation constants and RIE values were determined with the receptor alkylation method (Method 1, Furchgott, 1966; Furchgott and Bursztyn, 1967). Method 1 was only used with five partial agonists because alkylation of too large a fraction of the receptors often prevented weak partial agonists from eliciting a measurable response. Therefore, another method was required for determining the dissociation constants and RIE values of the partial agonists. The Barlow method (Method 2, Barlow et al., 1967), as modified by Kenakin and Black (1978), was used for all agonists with RIA values less than 0.9. Method 2 also provided an independent check of the results obtained with Method 1. The dissociation constants and RIE values obtained with either method were similar for five partial agonists. Therefore, for the sake of simplicity, no distinctions will be made as to which method was used to determine these values in the discussion of the structure-activity observations. Table 11, a distilled version of table 6, can be used to follow this discussion. From an examination of all the tryptamine analogs tested on the isolated rabbit aorta two important structure-activity relations became apparent.

First, methylation of the ethylamine side-chain group primarily influenced efficacy. As shown in each of the

five sets of tryptamine analogs in table 11, drugs with one or two methyl groups on the side-chain nitrogen had significantly lower intrinsic efficacy values than their respective primary amine analogs. This effect was most pronounced for the drugs possessing either a hydroxyl or ether substituent at the four position. For example, 4-HT and 4,5-MDOT were essentially full agonists but 4-HDMT and 4,5-MDODMT were partial agonists with one tenth the intrinsic efficacy of 5-HT. In the first three sets of tryptamine analogs (table 11) drugs with methyl groups on the side-chain nitrogen (5-HNMT, 5-HDMT, 5-MeODMT, NMT and DMT) had affinities similar (about two-fold differences) to their respective primary amine analogs (5-HT, 5-MeOT and T). However, in the two sets of tryptamine analogs with substituents at the four position drugs with two methyl groups on the side-chain nitrogen (4-HDMT and 4,5-MDODMT) had higher affinities (about six-fold differences) for the receptor than their respective primary amine analogs (4-HT, and 4,5-MDOT). It is possible that the dissociation constants of 4,5-MDODMT and 4-HDMT were overestimated with the modified Barlow method. The operational model of agonism (Black and Leff, 1983; Black et al., 1985) predicts that for steep CRCs the EC50 value for an agonist with low intrinsic efficacy is an overestimate of the dissociation constant. Therefore, the dissociation constants of 4-HDMT and 4,5-MDODMT were determined when these drugs were used as antagonists of

the response to 5-HT. Each of these drugs were simple competitive antagonists of the response (figs. 8 and 9). The dissociation constants of 4,5-MDODMT and 4-HDMT were 15 and 57 nM, respectively; the lowest of all the tryptamine analogs examined in this preparation. Therefore, the affinities of 4,5-MDODMT and 4-HDMT are actually about 16 times greater than those determined for 4,5MDOT and 4-HT. The reason(s) for the higher affinities for 4-HDMT and 4,5-MDODMT compared to 4-HT and 4,5-MDOT is not clear. However, it is interesting to note that the ergot alkaloids, e.g., d-lysergic acid diethylamide and methysergide which also have a high affinities for 5-HT_2 receptors (Leysen et al., 1982; Humphrey et al., 1982), are also N,N-dialkylated and four substituted tryptamine analogs. Tryptmine, AMT and alpha, alpha-dimethyltryptmine (A,ADMT) are another interesting set of drugs. The affinities of tryptamine and AMT are similar but, the efficacy of AMT appears to be less than that of tryptamine. Leff et al. (1986) reported that A,ADMT did not elicit a measurable contractile response in the isolated rabbit aorta and rat jugular vein. Consequently, A,ADMT was used as a competitive antagonist of the response to 5-HT in both preparations and its dissociation constant was about 2000 nM; similar to the values obtained for both tryptamine and AMT. Therefore, it seems that dimethylation of the alpha-carbon or the side-chain nitrogen has the same primary effect, the

reduction of efficacy. Finally, the removal of a single methylene group from the side-chain of 5-MeODMT would yield 5-MeOG. The dissociation constant of 5-MeOG is about five times greater than that of 5-MeODMT; however 5-MeOG has no observable efficacy and is a competitive antagonist of the response to 5-HT.

Second, some modifications of the tryptamine nucleus resulted in parallel increases and decreases in drug affinity (the reciprocal of the dissociation constant) and efficacy. Compared to tryptamine, the addition of: i) a methoxyl group at position five, ii) a hydroxyl group at positions four and five and iii) the addition of a methylenedioxyl group at positions four and five led to increases in drug affinity and efficacy. In contrast, the addition of a hydroxyl group at positions six and seven dramatically decreased affinity and efficacy. The inactivity of 7-HT does not seem to be due to steric hindrance created by the hydroxyl group at the seven position; the reason being that 7-MT is a partial agonist with an affinity and efficacy similar to those of tryptamine.

In summary, these results suggest that mono- and dimethylation of the side-chain nitrogen decreases intrinsic efficacy independent of the nature or position of substituents on the indole ring. For some tryptamine analogs the decrease in intrinsic efficacy is selective. However, the addition of two methyl groups had

differential effects for the analogs with either a hydroxyl or ether substituent at the four position. In both sets of four-substituted tryptamine analogs dimethylation decreased intrinsic efficacy and increased the affinity of the drug for the receptor. Taken together, the results in table 11 showed that methylation of the side-chain nitrogen dissociates affinity and intrinsic efficacy. Despite their low RIE values 4,5-MDODMT and 4-HDMT may serve as useful antagonists for the classification of 5-HT_2 receptors. It is interesting to note that competitive antagonists of the response to 5-HT were similarly developed from derivatives of 5-HT in other 5-HT-responsive systems. Offermeier and Ariens (1966) showed that some N-methyl, N-aralkyl derivatives of 5-HT were competitive antagonists in the isolated rat stomach fundus. Similarly, extensive modifications of the side-chain of 5-HT led to the development of a number of high affinity competitive antagonists in systems with peripheral neuronal (5-HT_3) receptors (Richardson et al., 1985). The integration of the results in this study with those reported by Leff et al. (1986) suggests that methylation of the alpha-carbon on the side-chain may have the same effect as that shown for the side-chain nitrogen. These contributions to the structure-activity relations of tryptamine analogs at 5-HT_2 receptors should be of value in understanding the structural requirements

necessary for the development of selective agonists and competitve antagonists with high affinity for this receptor.

II. Agonist and antagonist competition kinetics studies.

Isolated organ preparations are sensitive systems for measuring the consequences of drug-receptor interactions. These systems are used almost exclusively to obtain information about the relation between drug concentration and a pharmacological effect by measuring the effects at steady-state. However, the concentration-effect relation does not provide any information about the time-course of a response. Consequently, the details of drug-receptor interactions and the related molecular mechanisms of formation or inhibition of a response are obscured. Some of these details may be revealed in studies of the rate of action of agonists, and competitive and nonsurmountable antagonists.

Cory et al. (1984; 1986b) investigated the kinetics of agonist-induced contractions in isolated rabbit aorta and showed that: i) the rate determining step of the phasic response is the diffusion limited formation of the drug-receptor complex but ii) the rate determining step of the tonic response depends upon the concentration of the drug-receptor complex. Thus, the authors suggested that

the rate determining step of the tonic response in the rabbit aorta is the activation of an effector by the drug-receptor complex. The activation of the effector is loosely associated with the opening of calcium channels which is a necessary step for eliciting the contraction. In addition, Cory et al. (1986b) demonstrated that the maximal observable rate constant for the generation of the tonic response by the agonist, $k_{obs}max$, is a kinetic correlate of agonist efficacy.

The rate of onset of antagonism in various smooth muscle preparations was measured in earlier kinetic studies (Paton, 1961; Rang, 1966; Waud, 1967; Thron and Waud, 1968) by following the time dependent changes in responses elicited by low concentrations of agonist ([agonist]<<K_A) in the presence of an antagonist. The low concentrations of agonist minimized competition between the agonist and the antagonist for the receptor which allowed the simplifying assumption that the formation of the agonist-receptor complex does not influence the rate of onset of antagonism (Paton, 1961; Paton, 1975). Consequently, these studies only took account of the kinetics of the antagonist. With these methods Waud (1967) and Thron and Waud (1968) concluded that antagonist diffusion to the receptor through the biophase is the rate limiting process for the onset of antagonism. This conclusion implies that the rate of antagonist action cannot yield useful information about

the kinetics of antagonist-receptor interactions (Waud, 1967; Roberts and Stephenson, 1976).

This investigation was designed to show that the rate of antagonist action in isolated tissues can provide useful information about the kinetics of drug-receptor interactions. To achieve this goal an experimental protocol was devised to minimize the diffusion limitation in the intact tissue. A high concentration of the agonist, about 15 (5-HT) or 20 (tryptamine) times larger than the dissociation constant, was used to produce a steady-state response. At these concentrations the amount of free receptors, calculated from the equation $R_{free}/R_{total}=K_A/(A+K_A)$, was reduced to about 5 percent of the total population. Therefore, in the presence of the agonist, the rate of the antagonist-receptor interaction was decreased to the point where it became the rate limiting step. The onset of antagonism was monitored by a decrease in the response to the agonist. Consequently, a model describing the antagonist-induced decrease in the steady-state response had to take into account the interaction between the agonist and the receptor. The consequences of drug competition with the onset of antagonism were accounted for in equation 13, an analytical solution for the coupled differential equations that described the system of drug-receptor interactions.

The kinetics of two drugs competing for a single

recognition site were defined for radioligand binding sites (Motulski and Mahan, 1984; Schrieber et al., 1986a; 1986b). However, no attempt was made to study the kinetics of agonists and antagonists that compete for the same receptor in a functional system. The 5-HT receptor in the rabbit aorta is a good system to study the kinetics of drug competition for several reasons. First, the steady-state contractile response to an agonist is quite stable and reproducible. Second, the 5-HT receptors in this tissue represent a homogeneous population of 5-HT_2 receptors (Humphrey et al., 1982; Maayani et al., 1984). Finally, the actions of a number of serotonergic agonists (Clancy and Maayani, 1985) and competitive antagonists (Humphrey et al., 1982; Maayani et al., 1984) are defined in this preparation.

To test the validity of this method the following were examined: i) the effect of a diffusion barrier on the rate constants of the drugs; ii) the constancy of k_2, the dissociation rate constant of 5-HT, determined with the different antagonists and iii) the agreement between the ratio of the dissociation and the association rate constants and the equilibrium dissociation constants for a series of 5-HT_2 antagonists and for the agonist tryptamine.

To examine the effect of a diffusion barrier on the rate constants of the agonists and the antagonists, the kinetic assays were performed in the absence and in the

presence of the adventitia. The adventitia is the outermost of three layers of tissue that makeup the microscopic structure of arteries. In elastic arteries such as the aorta the adventitia consists of irregularly arranged connective tissue containing both collagenic and elastic fibers. Ruffolo and Patil (1979) studied the rate of onset of antagonism in isolated rabbit aorta and showed that the adventitia behaved as a diffusion barrier, limiting the rate of antagonist access to the receptors. The methods used by Ruffolo and Patil (1979) were similar to those used in earlier studies of the rate of onset of antagonism (Paton, 1961; Thron and Waud, 1967). If antagonist diffusion were rate limiting in the competition kinetics assays then one would expect significant differences in the estimates of agonist and antagonist rate constants determined in the absence and in the presence of the adventitia. The results showed that the estimated rate constants for the potent antagonists were similar for assays performed in the absence and in the presence of the adventitia. Thus, it was concluded that the presence of this diffusion barrier does not affect the kinetics of these specific drugs. However, the onset of antagonism of 5-MeOG was sensitive to the influence of this diffusion barrier. In the presence of the adventitia the average association and dissoiation rate constants of 5-MeOG were decreased by a factor of 4.5 as compared to the values obtained from assays performed in the absence

of the adventitia. The different behavior of the potent antagonists from that of 5-MeOG may be explained by the differences in their lipid solubility. The partition coefficients (log P) for ketanserin (Leysen et al., 1985), spiperone and methysergide are 3.3, 3.65 and 2.99, respectively. Therefore, their partitioning into the hydrophobic environment of the adventitia is preferred and their diffusion through this layer is rapid. Consequently, the absence or the presence of the adventitia would have little or no influence on the kinetics of these antagonists with our experimental design. By comparison, the log P for 5-MeOG is 1.18 and so 5-MeOG probably does not diffuse through the adventitia as readily as the three other antagonists.

The relatively unfavorable partition coefficient for 5-MeOG could account for the observed decrease in the kinetics of 5-MeOG in the presence of the adventitia. It is interesting to note that the adventitia has the same effect on both the association and the dissociation rate constants of 5-MeOG. The adventitia is as a hydrophobic barrier that separates the aqueous phase from the receptor. The decrease in the association rate constant (k_3) in the presence of the adventitia is due to a decrease in the rate of diffusion of 5-MeOG from the solution through the adventitia to the receptor. In the process of dissociation, 5-MeOG leaves the favorable environment of the receptor and diffuses into the

unfavorable environment of the adventitia. Thus, the adventitia would also decrease the dissociaion rate constant (k_4). Because the adventitia has approximately the same effect on both the association and the dissociaton rate constants it has no observable influence on the steady-state dissociation constant of the antagonist.

In each experiment two assays were performed on a given ring of the aorta. The data from these assays were compiled and fitted simultaneously to obtain estimates of three rate constants, e.g. k_2, k_3 and k_4. In this way the information in each fit was maximized and the fit of the model to one assay was constrained by the information contained in the other assay. The high quality of the curve fitting (r^2=0.99) indicated that the rate constants were independent of antagonist concentration. Furthermore, despite the use of different antagonists, over wide ranges of concentration, the dissociation rate constant of 5-HT remained essentially the same (table 7). Thus, it was concluded that the kinetics of the agonist are independent of the antagonist used. Similarly, the results with spiperone suggested that the kinetics of the antagonist were independent of the agonist used (table 7). It is important to note that the values of the three kinetic rate constants determined in these assays depend upon the value of the dissociation constant of either the agonist or the antagonist. For

example, the dissociation constant of either 5-HT or tryptamine was substituted into equation 13 to obtain an estimate of the association rate constant of spiperone. Perhaps the almost two fold difference in the values of k_3 for spiperone (table 7) can be explained by the small degree of uncertainty associated with the values of the dissociation constants of 5-HT and tryptamine (Clancy and Maayani, 1985).

The validity of the kinetic analysis can also be evaluated from the data in tables 7 and 8 which show that the dissociation constants calculated for tryptamine and the antagonists are similar to their respective dissociation constants determined with steady-state methods. Thus, as expected, when the system reaches steady-state, a dynamic equilibrium is established and the association rate equals the dissociation rate. This would suggest that the kinetic rate constants reflect the molecular events in the interaction of the drugs with the receptor. This conclusion is further supported by the excellent agreement (table 7) between the kinetic rate constants estimated for the potent antagonists in the rabbit aorta and those reported in high affinity binding experiments (Leysen and Gommeren, 1986).

An interesting relation exists between the rate constants of a drug and its affinity for the 5-HT_2 receptor. The association rate constants (k_3) of the high affinity antagonists, i.e. spiperone, ketanserin and

methysergide, are about two orders of magnitude greater than that estimated for 5-MeOG. The dissociation rate constants (k_4) of all the antagonists are of a similar order of magnitude (see tables 1 and 2). Similarly, the average calculated value of the association rate constant of 5-HT (k_1) is 2.27+/-0.37 $(uMmin)^{-1}$, about one order of magnitude greater than that determined for tryptamine. The dissociation rate constants (k_2) for both agonists are about the same (table 7). The difference in k_1 between 5-HT and tryptamine is quantitatively consistent with the difference in their dissociation constants. Thus, it appears that the association rate constant is an important determinant of drug affinity for the receptor. This is consistent with observations made by Contreras et al. (1986) on the binding of various agonists and antagonists to beta-adrenergic receptors. These observations contradict the suggestion that the dissociation rate constant is the primary determinant of drug affinity for a receptor, i.e. k_{-x} is directly proportional to the drug-receptor dissociation constant (van Ginneken, 1977).

In summary, the experimental design presented here enabled the investigation of the kinetics of simple agonist and antagonist competition in an intact preparation. The results indicate that the estimated rate constants reflect molecular events in the interaction of the drugs with the receptor. The relationship between the

kinetic rate constants and the equilibrium dissociation constant reveals that the association rate constant is a primary determinant of drug affinity for the receptor. Finally, the ratio of the kinetic rate constants can be used to evaluate the equilibrium dissociation constant regardless of the presence of the adventitia which acts as a symmetric diffusion barrier. It is possible the a kinetic analysis of this kind may contribute additional insight into the mechanism of action of complex drug actions, e.g., nonsurmountable antagonism. Nonsurmountable antagonism is usually investigated with steady-state methods and little is known about the time-dependent effects of these drugs.

III. Nonsurmountable antagonism produced by LSD and IOL.

LSD and IOL were nonsurmountable antagonists of the response to 5-HT. Three charcteristics of the antagonism were observed. In the presence of either antagonist, the maximal response was decreased, the CRC was displaced to the right in a nonparallel manner and the rate of the response was slower. These effects were selective for the response to 5-HT, i.e., the responses to histamine, phenylephrine and KCl were not affected, suggesting that LSD and IOL do not act as functional or physiological antagonists of the contractile response. In support of

this suggestion, the removal of endothelial cells from the tissue or the presence of 3 uM methylene blue did not affect the parameters of the CRC (equation 1) in the absence or in the presence of 10 nM LSD. These results suggest that the basal concentration of cGMP (under these experimental conditions) does not influence the response to 5-HT. Moreover, the nonsurmountable antagonism produced by LSD does not appear to be due to a cGMP-mediated relaxation of the tissue. Since the antagonist effects of LSD and IOL were qualitatively similar (fig. 16) their mechanism of action may be the same. With this assumption we attempted to study the components of the antagonism produced by LSD or IOL in order to understand the interactions of these drugs with 5-HT_2 receptors.

In the presence of increasing concentrations of IOL the rightward shifts of the CRC (fig. 17) indicated that IOL may act in part as a competitive antagonist of the response to 5-HT. The concentration ratios obtained in the presence of IOL and spiperone were additive (table 9) but were greater than expected. This discrepancy can be attributed to the concentration ratio obtained in the presence of IOL. Leff and Morse (1987) showed that the rightward displacements of the CRC produced by a nonsurmountable, competitive antagonist can be greater than expected due to the decrease in the maximal response. Therefore, it is possible that the observed concentration

ratio obtained in the presence of IOL is an overestimate. Despite this complication the demonstration of antagonist additivity implies that IOL binds to the 5-HT-recognition site on 5-HT_2 receptors.

The decreases in the maximal response and in the rate of the response to 5-HT produced by LSD and IOL were prevented by exposing the tissue to a competitive antagonist (fig. 16 and table 10). These results suggest that these two antagonist effects occur as a consequence of the binding of LSD and IOL to the 5-HT-recognition site on 5-HT_2 receptors. Studies of the relation between the concentration of LSD and the decrease in the maximal response provided a quantitative test of this hypothesis. Ketanserin and 5-MeOG were selected as antagonists of the LSD effect because their structures and affinities for the 5-HT_2 receptor are different. If this hypothesis is valid then the CRC of LSD will be shifted to the right in the presence of the competitive antagonists. In addition, the dissociation constants of ketanserin and 5-MeOG will be equal to those constants measured when these drugs antagonize the response to 5-HT. The results in figure 18 support this hypothesis. Taken together, the steady-state experiments indicate that all characteristics of the nonsurmountable antagonism produced by LSD and IOL occur as a consequence of antagonist binding to the 5-HT-recognition site on 5-HT_2 receptors. The competitive antagonism produced by LSD and IOL occurs as

part of a pharmacological resultant as defined by Black et al. (1986). In this case, the competitive component appears to occur in combination with another receptor-dependent action of the antagonists.

It does not appear that LSD and IOL slowly dissociate from 5-HT_2 receptors. To probe this hypothesis a model was developed to analyze the kinetics of agonist and antagonist competition for the same receptor. This model is useful because the estimated rate constants reflect primarily the molecular interactions of these drugs with the receptor. If the effects of LSD and IOL are due to their slow dissociation from the receptor, then their interaction with the receptor should be the same as that assumed for a surmountable, competitive antagonists, i.e., $B + R \underset{k_{-X}}{\overset{k_X}{\rightleftharpoons}} BR$. Therefore, analysis of the onset of nonsurmountable antagonism with this model (equation 13) would be appropriate. The steady-state dissociation constants of LSD (Leysen et al., 1982) and IOL (Engel et al., 1984; Kadan et al., 1984) are similar to those of spiperone, ketanserin and methysergide (Leysen et al., 1982) at 5-HT_2 binding sites. If LSD and IOL slowly dissociate from the receptor, compared to the other three antagonists, then their rate of association with the receptor will be slower by the same factor such that the ratio of the kinetic rate constants (k_{-X}/k_X) equals the dissociation constant. Consequently, the rate of decrease in the steady-state response to 5-HT after the

addition of LSD or IOL should be slower than that observed for similar concentrations of an equally potent competitive antagonist, e.g., methysergide. To illustrate this point, simulations of the onset of antagonism of three different antagonists are shown in figure 23. In this figure the antagonists have a dissociation constant of 1 nM but differ in their rates of association with and dissociation from the receptor. The onset of antagonism produced by LSD and IOL (figs. 20 and 21) was not adequately described by equation 13; the fit was poor and the parameter estimates were not physiologically meaningful. The onset of antagonism appeared sigmoidal and as fast as that observed with similar concentrations of methysergide (fig. 19). These observations strongly suggest that the nonsurmountable antagonism produced by LSD and IOL is not due to their slow association with and dissociation from the 5-HT_2 receptor.

Black et al. (1983) reported that LSD was a contractile agonist in the isolated rabbit aorta. In addition, they showed that the response to LSD was prevented by prior exposure of the tissue to methysergide or trazodone which were competitive antagonists of the response to 5-HT in the rabbit aorta (Leff and Martin, 1986). Bay K 8644, a calcium channel agonist, has been shown to increase the efficacy of 5-HT in the isolated rabbit aorta without changing agonist or antagonist affinity for 5-HT_2 receptors (Barrett et al., 1986).

LSD and IOL were tested in the presence of 300 nM Bay K 8644 in order to obtain consistent contractile effects with LSD and to examine IOL as a potential agonist in a more sensitive system. In the presence of Bay K 8644 the intrinsic activity of 20 nM LSD, relative to the maximal response to 5-HT, was 0.52. Prior exposure of the tissues to 200 nM spiperone blocked the response to LSD. This observation, together with those reported by Black et al. (1983), indicated that the response to LSD was mediated by 5-HT_2 receptors. An intriguing observation was made when the order of spiperone and LSD administration was reversed. In competition kinetics assays with 20 nM LSD (about 10 K_D, Leysen et al., 1982) as the agonist, the addition of 200 nM spiperone had little if any effect on the steady-state response (fig. 22B). In competition kinetics assays with 3 uM 5-HT (about 15 K_D) as the agonist, the addition of a competitve antagonist resulted in an immediate decrease in the steady-state response (figs. 12, 13, 14, 15 and 19). These results suggested that LSD was an atypical agonist since it was able to elicit an effect that was not readily decreased after the addition of a competitive antagonist.

Taken together, all the results lead one to suggest that the LSD and IOL antagonist-receptor complexes interact with an element of the signal transducer system in an undefined manner. One possibility could be that a ternary complex is formed as follows:

rapid slow

$$B + R \underset{K_D}{\rightleftharpoons} BR + T \underset{K_T}{\rightleftharpoons} BRT$$

where B is the nonsurmountable antagonist, R is the receptor, T is an undefined transducer molecule, BRT is the ternary complex and K_D and K_T are the dissociation constants for BR and BRT, respectively. The observation that LSD was an agonist at 5-HT_2 receptors (Black et al., 1983; Laubscher et al., 1981; Pletscher and Affolter, 1983) supports the suggestion that BRT can be formed in a manner consistent with the operational model of pharmacological agonism (Black and Leff, 1983). BRT may be more stable than the ternary complex formed in the presence of a typical agonist such as 5-HT, i.e., the formation of BRT is slowly reversible. This hypothesis could account for several experimental observations. First, LSD and IOL are nonsurmountable, competitive antagonists of the response to 5-HT. The formation of BRT would effectively remove receptors and transducer elements from the system such that increasing concentrations of agonist may not surmount the presence of the antagonist during the time agonist is exposed to the system. Second, equilibration of the system with a surmountable, competitive antagonist protects the maximal response to 5HT. With two antagonists the concentration

of receptors occupied by the nonsurmountable antagonist (BR) would be less than when the nonsurmountable antagonist is present alone. The decrease in BR decreases BRT which increases the concentration of receptors and transducers available to the agonist. Third, the onset of the antagonism of LSD and IOL is rapid and appears sigmoidal. After the nonsurmountable antagonist is added at time zero there is a brief and linear decrease in the steady-state response to 5-HT. This phase of the onset can be attributed to the rapid formation of BR. The concentration of BR accumulates until it can effectively interact with the transducer element to form a ternary complex. This process removes receptors and transducers from the system with the loss of either or both resulting in a more rapid decrease in the response. Therefore, the sequential steps in the formation of BRT result in the sigmoidal onset of antagonism. Fourth, 20 nM LSD induces a steady-state contractile response in the rabbit aorta that is insensitive to the addition of 200 nM spiperone over the time of the assay depictd in figure 22B. Presumably, spiperone equilibrates with the receptors rapidly and reduces the steady-state concentration of BR. However, the concentration of BRT and the associated response are reduced slowly during the time of exposure to spiperone. Fifth, IOL is not an agonist. This implies that there are additional steps beyond the formation of BRT which lead to a response. The BRT complexes formed by

IOL and LSD may differ in their ability to stimulate the system. The observation that IOL decreases the response to 5-HT less than does LSD could be explained by differenes in the value of K_T. If K_T(IOL) is larger than K_T(LSD), then for equal concentrations of BR there is less BRT(IOL) than BRT(LSD).

This model and the one proposed by De Chaffoy de Courcelles et al. (1986) are similar in that they introduce the concept of receptor-transducer coupling to explain the effects of nonsurmountable antagonists. However, there are important differences between these models. According to their model, competitive and nonsurmountable antagonists decrease the probability of an interaction between the agonist-receptor complex and the transducer, i.e., all antagonists reduce agonist efficacy. This assumption is not required in the model proposed here. In the case of nonsurmountable antagonism their model predicts that the formation of an antagonist-receptor complex changes Sin such that coupling with the transducer is impossible. Therefore, in their model it would be unlikely that a ternary complex would be formed from an interaction between the antagonist, the receptor and the transducer. This would imply the nonsurmountable partial agonists such as LSD do not use the same transducer element used by the natural or a typical agonist.

In summary, LSD and IOL are nonsurmountable

antagonists of the response to 5-HT in the rabbit aorta. Steady-state and kinetic measurements indicated that all characteristics of the nonsurmountable antagonism or agonism occur as a consequence of LSD and IOL binding to the 5-HT-recognition site on 5-HT_2 receptors. It was hypothesized that the antagonist-receptor complex may interact with an element of the transducer system in an undefined manner, possibily to form a stable (slowly reversible) ternary complex. Such interactions may also explain the slow kinetics of LSD dissociation from 5-HT_2 binding sites in tissue homogenates (Geaney et al., 1984; Leysen et al., 1986).

IV. Summary

The 5-HT_2 receptor in the isolated rabbit aorta was characterized with eighteen tryptamine analogs and four simple competitive and two nonsurmountable antagonists of the response to 5-HT. Drug-receptor interactions were investigated with established steady-state methods and a novel kinetics method.

1. Structure-activity observations of the tryptamine analogs revealed some structural requirements necessary for the deveolpment of competitive antagonists, of reasonably high affinity, and possibly selective agonists for the 5-HT_2 receptor. It is possible that these agonists and antagonists may be useful in the

classification of 5-HT receptors without the complications that have been reported for some conventional antagonists such as ketanserin, methysergide and spiperone.

2. A novel kinetics method was developed in a functional preparation for the simultaneous analysis of agonist and antagonist competition for the receptor. The results indicated that the estimated rate constants reflect molecular events in the interaction of the drugs with the receptor. The relationship between the kinetic rate constants and the equilibrium dissociation constant revealed that the association rate constant was a primary determinant of drug affinity for the receptor. This method can be used with ease to provide accurate determinations of dissociation constants of typical agonists and competitive antagonists; an alternative for measuring the agonist dissociation constant when an alkylating agent is not available or desired.

3. Steady-state and competition kinetics measurements indicated that all characteristcs of the nonsurmountable antagonism produced by LSD and IOL occured as a consequence of antagonist binding to the 5-HT-recognition site on 5-HT_2 receptors. It was hypothesized that the antagonist-receptor complex may interact with some element of the transducer system in an undefined manner, possibly to form a stable (slowly reversible) ternary complex. Additional studies of these kinds of nonsurmountable antagonists will further our understanding of the

interactions of drug-receptor complexes with the effector system.

Table 11. Dissociation constants and relative intrinsic efficacy values of the tryptamine analogs at 5-HT_2 receptors in the isolated rabbit aorta.

Drug	K_A (nM)[a]	RIE[b]	N[c]
5-HT[d]	250+/-20	1	7
5-HNMT	180+/-30	0.24+/-0.03	3
5-HDMT	110+/-20	0.20+/-0.01	4
5-MeOT[d]	140+/-40	0.86+/-0.12	5,3
5-MeODMT	125+/-10	0.21+/-0.02	6
T	1090+/-70	0.48+/-0.06	5
NMT	1060+/-170	0.17+/-0.02	4
DMT	506+/-167	0.16+/-0.02	5
4-HT[d]	875+/-260	0.81+/-0.06	4,5
4-HDMT	146+/-38	0.08+/-0.01	3
4,5-MDOT[d]	276+/-11	1.21+/-0.20	4,5
4,5-MDODMT	50+/-8	0.07+/-0.02	3

Continued on next page.

Table 11 continued.

Drug	K_A (nM)	RIE	N
5-MT	530+/-80	0.34+/-0.07	4
7-MT	1280+/-220	0.35+/-0.07	3
AMT	1900+/-200	0.25+/-0.03	4
A,ADMT[e]	2140	0	6
5-MeOG[f]	616	0	2
6-HT	N.D.	N.D.	
7-HT	N.D.	N.D.	

[a] Values are the geometric mean +/- S.E.M.

[b] Values are the arithmetic mean +/- S.E.M.

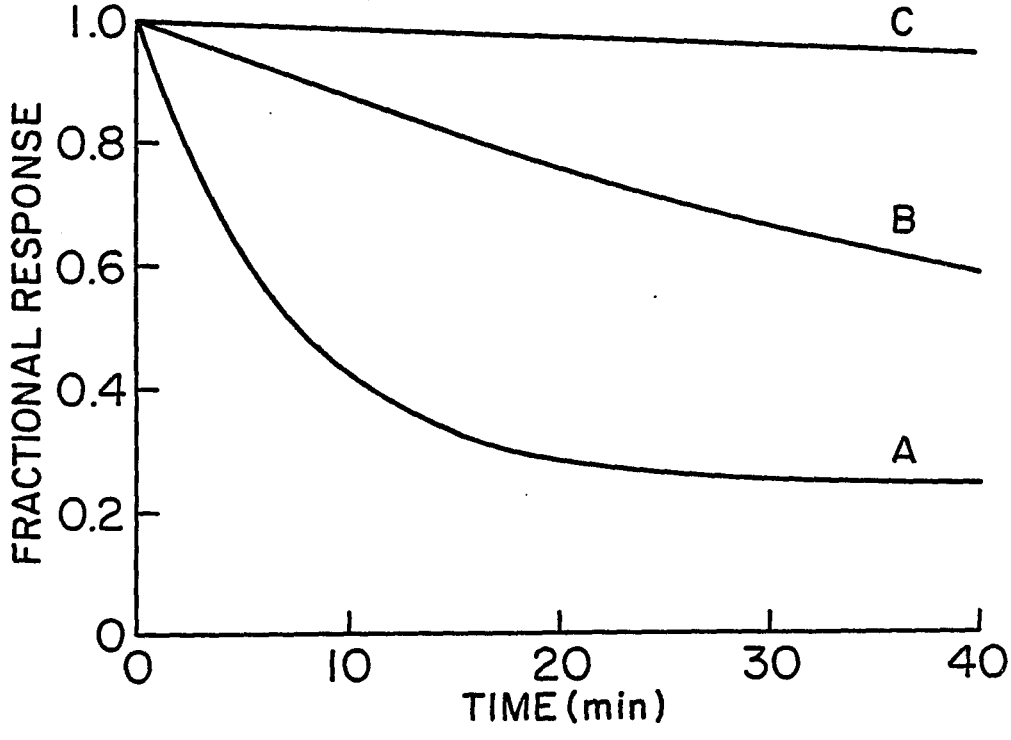
[c] Number of rabbits used.

[d] Determined with the method of Furchgott and Buraztyn (1967). Parameter estimates for the other drugs were determined with the method of Barlow et al. (1967) as modified by Kenakin and Black (1978).

[e] Data taken from Leff et al. (1986).

[f] Determined in this study; data derived from a Schild plot

Figure 23. Simulations of the competition kinetics model (equation 13) for three competitive antagonists of the response to 5-HT in the isolated rabbit aorta. In each curve k_2=0.35 min^{-1}; k_2/k_1=0.2 uM; [agonist]=3 uM; [antagonist]=50 nM and k_4/k_3=1 nM. A. k_3=50 $(uMmin)^{-1}$ and k_4=0.05 min^{-1}. B. k_3=5 $(uMmin)^{-1}$ and k_4=0.005 min^{-1}. C. k_3=0.5 $(uMmin)^{-1}$ and k_4=0.0005 min^{-1}.


1.0
0.8
0.6
0.4
0.2
0
FRACTIONAL RESPONSE
C
B
A
0
10
20
30
40
TIME (min)

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