DRUGS AFFECTING SMOOTH MUSCLE[1,2]

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While it is tempting and often desirable to regard smooth muscle as a single entity, different types have anatomical, physiological, and pharmacological differences. In this review of the recent literature on the effect of drugs on various kinds of mammalian smooth muscle the mechanism of drug action has been emphasized when possible and studies of drug-receptor interactions in smooth muscle have been examined. Page limitations prevented citing many worthy investigations but the papers selected, in our opinion, contained promising findings apt to influence future studies of drugs affecting smooth muscle. Past reviews in this series have emphasized the effects of drugs on electrical activity or the role of various ions in the response mechanism. Our approach has been to write a more general review.

Vascular Muscle

Pardo, Hong & LeLorier (1) have examined the phenomenon of epinephrine autoinhibition in rabbit aortic strips. Autoinhibition was observed even at extremely low concentrations, and the intensity and duration of inhibition were greater after higher concentrations of epinephrine. The modification of dose-response relationships by variation of the time interval between successive contacts was also demonstrated. Interestingly, other authors (2) have demonstrated potentiation of the effects of low concentrations of epinephrine by higher concentrations. Full potentiation develops slowly and the effect seems remarkably specific for epinephrine. Tension differences or other variations in experimental technique may account for the differences in observations here and elsewhere (1, 3).

Preparations of perfused isolated branches of the dog femoral artery (4) or the central artery of the rabbit's ear (5–11) have recently been employed to study the interactions of various autonomic agents. Carrier & Holland (4), investigating the phenomenon of reserpine-induced supersensitivity to norepinephrine, found that different routes of drug administration led to quantitatively different results. The response of reserpinized small ar-

[1] The survey of literature pertaining to this review was concluded in June 1968.
[2] The following abbreviations are used in this review:
BOL (2-bromolysergic acid diethylamide),
DCI [1-(3,4-dichlorophenyl)-2-isopropylaminoethanol],
MJ1999 [4-(2-isopropylamino-1-hydroxyethyl)methanesulfonanilide HCl].

teries to perfusion with norepinephrine was the same as the response of controls. However, after an injection of norepinephrine, potentiation and a lower dose threshold were observed in reserpinized preparations. The possibility of repletion of the norepinephrine stores by the perfusion method was considered. Based on an analysis of rates of response of the two preparations, the authors suggest that reserpine increases the effective receptor areas on the effector organ either functionally or anatomically. If this were so, then the maximum response to norepinephrine should be increased in the reserpine-treated preparations but since the authors did not attempt to obtain maximum responses, this conclusion should remain tentative.

The isolated innervated central artery preparation (5) responds to a variety of agents and procedures. Vasoconstriction can be induced by stimulation applied transmurally, peri-arterially, or to the ventral auricular nerve, and the response is enhanced after cocaine and abolished or greatly reduced after guanethidine, procaine, or phenoxybenzamine. Norepinephrine- or isoproterenol-induced (11) vasoconstriction was enhanced by cocaine and blocked by phenoxybenzamine. Vasodilatation following acetylcholine was observed only in the presence of increased sypathetic tone and was greatly reduced by atropine, while vasodilatation following isoproterenol was never observed. Gay, Rand & Wilson have, therefore, concluded that beta receptors are absent in this preparation (11).

Serotonin, while only weakly active itself, markedly potentiated the action of norepinephrine and sympathetic nerve stimulation on the central artery. This interesting interaction was further investigated (7) and found to occur both in the absence and presence of a constrictor response to serotonin. The sensitization is apparently nonspecific since the constrictor response to histamine and angiotensin was also enhanced and sensitization to histamine still occurred in the phenoxybenzamine-treated (7) and chronically sympathectomized (5) artery. The sensitizing action of serotonin is shared by one serotonin antagonist (methysergide) but blocked by another (BOL). It is suggested that serotonin's action in this preparation is mediated via receptors analogous to serotonin receptors in other types of smooth muscle.

Tyramine, which acts indirectly, produces a biphasic vasoconstriction of the central artery preparation (6), the first phase being short lived and similar to that produced by norepinephrine, followed by a second phase of similar height but longer duration. The first phase of the response to tyramine was not markedly altered by cocaine, reserpine pretreatment, or sympathetic denervation (like norepinephrine) but the second phase was reduced or abolished by these treatments and restored by perfusion with norepinephrine in reserpine-pretreated, but not denervated, preparations. Phentolamine shifted the dose-response curve for norepinephrine and the second phase of tyramine to the right but had much less effect on the first phase. The first phase is therefore probably the result of direct alpha receptor stimulation by tyramine acting as a partial agonist and the second phase is probably due

to release of catecholamines from intraneuronal stores. These results have recently been extended (10) to include other sympathomimetic amines; octopamine, phenylethylamine, and phenylethanolamine. All behaved qualitatively like tyramine and were similarly affected by denervation, reserpine, and cocaine treatment.

The central artery preparation is much less sensitive to extra-luminal than to intra-luminal norepinephrine (7). Cocaine applied either intra- or extra-luminally abolishes this difference in sensitivity (9), a finding consistent with the assumption that uptake plays a principal role in the response of the artery to norepinephrine. If the sites of uptake are distributed around the outer perimeter of the smooth muscle and are equally accessible to norepinephrine applied to either side of the artery, then norepinephrine applied from the adventitial side (extra-luminally) is exposed to these sites before it enters the smooth muscle layer and acts. Norepinephrine applied to the intima (intra-luminally) is exposed to uptake sites only after it has diffused through the muscle layer so that uptake has far less effect. If uptake is blocked by cocaine, then the response of the preparation to extra- and intra-luminal norepinephrine should be the same, as has been observed. Catecholamine-like fluorescence was found only in the layer immediately outside the circular smooth muscle, between this and the adventitia; an anatomical confirmation of the pharmacological observations. Avakian & Gillespie (8), also employing fluorescence techniques, have suggested that both smooth muscle and adrenergic nerves of these arteries can take up catecholamines intracellularly and that smooth muscle is responsible for uptake at high concentrations ($Uptake_2$).[3]

Bevan & Verity (12) and Maxwell, Eckhardt & Wastila (13) using two nerve-free aortic preparations have attempted to assess the importance of uptake as a mechanism for the disposition of sympathetic transmitter. In both preparations, complete denervation was shown either histologically, or by the failure of response to transmural stimulation, or both. These preparations showed marked subsensitivity to tyramine (12, 13), loss of response to nicotine (12), and a reduction in the maximum response to norepinephrine (12, 13), without a change in the median effective dose, slope, or shape of the dose-response curve or time to equilibrium (12). Cocaine has no effect on the dose-response curve in these denervated preparations although it caused a shift in the dose-response curve to norepinephrine to the left and increased the response to transmural stimulation. It did cause an increase in the maximum response to norepinephrine in both normal and denervated preparations, possibly due to both a presynaptic and a direct action of cocaine on the smooth muscle (12).[3] Maxwell (13) provided additional evidence that the major portion of the sympathetic innervation of the rabbit aorta is restricted to the adventitia, which contains 75 per cent of the endogenous norepinephrine content of the aorta. The adventitia also has 80 to

[3] For an alternative explanation based on prevention of autoinhibition by cocaine, see VAS DEFERENS section.

90 per cent of the norepinephrine binding capacity of the aorta, with the binding being sensitive to inhibition by cocaine. The remaining 10 to 20 per cent binding capacity of the medial-intimal layers is cocaine-insensitive. The authors conclude from this and other work (14) that cocaine enhances the response of aortic strips to norepinephrine by a direct action on smooth muscle and that, further, it is unlikely that binding of norepinephrine by the relatively sparse innervation of the aorta or other blood vessels can effectively regulate the concentration in the media. This contrasts with the opinion of de la Lande & Waterson (9) who consider the occurrence of uptake sites on the outer perimeter of the smooth muscle layer to have *in vivo* significance, and the recent contribution of Gerová, Gero & Doležel (15) which presents evidence that catecholamines released by sympathetic stimulation can diffuse from nerve endings to smooth muscle layers remote from the nerve terminals.

Two recent studies discuss the use of other preparations for the study of drug action on uninnervated smooth muscle: that of the umbilical artery (16, 17) and vein (16). The preparations exhibit rhythmic, spontaneous contractions and differing amounts of tone. Acetylcholine produces either a constrictor or a dilator response which is blocked by atropine or hyoscine but not modified by physostigmine. Serotonin, histamine, and bradykinin produce vasoconstrictor responses which are specifically blocked by BOL or cyproheptadine, antazoline or mepyramine, and acetyl salicylic acid, respectively (17). Tachyphylaxis was not observed to the action of serotonin as occurs with some other preparations. Oxytocin was found to be an extremely potent vasoconstrictor by Somlyo, Woo & Somlyo (16) while the response to vasopressin and angiotensin was strangely absent. Extremely variable responses to catecholamines occurred at higher concentrations than in somatic arterial preparations. Constrictor and dilator responses to epinephrine and norepinephrine were blocked by phentolamine, dihydroergotamine (17), or dibenamine (16), but not by pronethalol (17). In addition, isoproterenol did not induce vasodilation in resting preparations, following serotonin- or histamine-induced contraction, or after dibenamine blockade. High concentrations produced variable vasoconstriction (16). Thus, the absence of beta adrenergic receptors seems certain in this preparation. Cocaine not only fails to potentiate the effects of norepinephrine (which is expected in the absence of nerves) but in most cases depresses its effects, an action difficult to reconcile with the above-mentioned hypothesis (12–14) of a direct sensitizing effect on smooth muscle.

A comparative phylogenetic study of the pharmacology of arterial strips has produced some interesting observations (18). Spiral strips were prepared from systemic arteries of a reptile and an amphibian, and from the ventral aorta of two teleosts. Although differing in threshold sensitivity, all arterial strips contracted in response to norepinephrine and epinephrine, and the effects of these agents could be blocked by phentolamine, phenoxybenzamine, and dibenamine in all preparations. Isoproterenol caused only

contraction of the reptilian aorta and had no effect over a 10,000-fold concentration range on the amphibian or teleost arteries, even when the strips were contracted in response to acetylcholine or epinephrine. In spite of the apparent absence of beta inhibitory receptors in these vessels pronethalol in small concentrations blocked the contractile actions of catecholamines on the reptilian and amphibian preparations with variable results on the teleosts. Thus, its effects here may be nonspecific.

Many attempts have been made to explain why dichloroisoproterenol (DCI) (19, 20), pronethalol (20–23), and propranolol (24, 25) reverse the adrenergic vasodepression seen after the administration of alpha-receptor blocking agents. In typical experiments, after control dose-response curves are obtained, phenoxybenzamine (19–25), dihydroergotamine (19, 23), hydergine (19) tolazoline (24), phentolamine (24, 23), or benzodioxane (19) is administered to cause adrenergic blockade and unmask the vasodilator effects of epinephrine. Subsequently, a beta blocker is given and the response to catecholamines becomes pressor (or vasoconstrictor) again although never reaching the control levels in response. Two explanations have been advanced for these observations. The first involves a direct "deblocking" or "unblocking" effect of the beta receptor antagonist on the alpha receptor. Such a mechanism has been proposed on the basis of *in vitro* experiments with rabbit aorta. Gulati, Gokhale & Udwadia (23) showed the antagonism of phentolamine blockade by pronethalol is competitive, implying that pronethalol has alpha-blocking properties (cf. 18). Olivares, Smith & Aronow (24) showed slight reversal of phenoxybenzamine blockade by propranolol which also interfered with the establishment of blockade in one experiment. DCI does not prevent the establishment of blockade *in vivo* (20), although propranolol was reported to attenuate its development in one experiment (24). Gay, Rand & Wilson (11) have also attributed weak alpha blocking properties to propanolol in high doses. The *in vivo* significance of this effect, however, remains obscure. An alternative explanation, preferred by most authors (20–22, 25) is based upon the hypothesis of Nickerson, Henry, & Nomaguchi (26) who concluded that adrenergic blockade by beta haloalkylamines causes vasomotor reversal when only a part (up to 50 per cent) of the alpha receptors are occupied. Therefore, the action of epinephrine is reversed because the beta receptors are more numerous than the remainder of the alpha receptors, or the amine has a lower affinity for these latter or both. Following beta receptor blocking agents, the injected catecholamine can react only with the alpha receptors not occupied by phenoxybenzamine. Thus, the action of epinephrine would depend upon the "balance" of receptors at any one time. Additional support for this view is provided by the finding that benzodioxane 883F regularly abolishes the pronethalol-induced reversal (22).

The differentiation of serotonin receptors in rabbit aorta from those for catecholamines and histamine has been attempted by Wurzel (27) and Kohli (28) using specific receptor antagonists. These studies have shown

that the receptors for serotonin can be selectively and reversibly antagonized by appropriate concentrations of BAS (1-benzyl-2-methyl-5-methoxytryptamine), BAS-phenol (1-benzyl-2-methyl-5-hydroxytryptamine), DMBC (n-*beta*-dimethylaminoethyl-N-benzyl-*m*-methoxy cinnamamide), (27) or BOL (28) but not by mepyramine, morphine, and LSD (27). Nonselective blockade was shown by phenoxybenzamine and DCI (27) as shown by similar I_{50} concentrations against all three agonists. In addition, Kohli (28), in comparing the pA_{10} values for phentolamine and BOL against a series of sympathomimetic amines and serotonin, concluded that all the sympathomimetics acted on norepinephrine receptors. The possible pitfalls in the use of the "cross-protection" technique are also discussed.

Wohl, Hausler & Roth (29) have attempted to elucidate the mechanism by which diazoxide produces its anti-hypertensive effects. Utilizing an *in vitro* approach based upon Lineweaver-Burk plots and pA_2-pA_{10} values, they present evidence that diazoxide acts at the same receptor site in rat aorta as Ba^{++}, in a way analogous to the competitive interactions of phentolamine and norepinephrine at the alpha adrenergic receptor. It was suggested that this site may normally be activated by Ca^{++}. Such an action, closely related to muscle contraction, could explain the ability of this class of agents to suppress pressor responses to a variety of vasoconstrictors.

The existence of two mechanisms of action of angiotensin on the isolated aorta of guinea pigs and rats was shown by a variety of methods (30). Contractions in response to angiotensin II were reduced by cocaine, phentolamine, and repeated doses of tyramine. Also, the action of angiotensin in preparations from reserpine-pretreated or alpha-methyldopa-pretreated animals is appropriately reduced. The results suggest that about two-thirds of the angiotensin-induced contraction is indirectly mediated through catecholamine release.

The interactions of anti-inflammatory drugs with a variety of vasoactive amines and peptides have been studied on various smooth muscle preparations from different species (31, 32). The vasoconstrictor response of several isolated perfused arterial (31, 32) and venous (31) segments was reduced by drugs of the analgesic-antipyretic type, and the degree of antagonism observed was independent of the nature of the constricting agent, i.e., nonspecific. Venous muscle tended to be more sensitive than arterial muscle to the more potent antiphlogistic agents but both were equisensitive to the less potent drugs. The response of other smooth muscle [ileum, vas deferens (21)], however, was far less sensitive to inhibition by these agents than vascular muscle. The concentrations of anti-inflammatory drugs required to antagonize vascular constriction *in vitro* are within the plasma concentrations attained *in vivo* after doses sufficient to produce anti-inflammatory effects when given to animals. This finding is especially intriguing.

The cardiovascular actions of prostaglandins are well reviewed in a recent article (33) and will not be dealt with here.

The pharmacology of venous smooth muscle has been recently inves-

TABLE I

CHARACTERISTICS OF SOME ISOLATED VENOUS SEGMENTS AND THEIR RESPONSES TO SELECTED AGONISTS

	External Jugular Vein	Inferior Vena Cava		Superior Vena Cava	Anterior Mesenteric or Portal Vein	Pulmonary Vein
	Rabbit (35)	Rabbit (36)[a]	Rat (36)[b]	Rat (36)	Rabbit (35, 37)	Rat (36)
Rhythmicity	Absent	Absent	Present	Absent	Present	Absent
Histamine	+	+	0	0	+	0
Norepinephrine	+	+	+	+	+	+
Epinephrine	+	+	+	+	+	+
Isoproterenol	0	+	+	+	[c]	+
Acetylcholine	0	+	−	−	+	−
Serotonin	0	0	0	0	+	0
Angiotensin	+	+	0	0	+	0
Vasopressin	0	0	?	?	[d]	?
Bradykinin	?	?	?	?	+	?
Oxytocin	?	?	0	0	−	0
Adenine Nucleotides	?	?	?	?	−	?

Explanation of symbols: + = contraction; − = relaxation; 0 = no effect; and ? = not studied.

[a] From below renal veins.

[b] Near heart.

[c] Low doses relax, high doses contract (35).

[d] No effect (35), relaxation (37).

tiated by several workers (34–37). Isolated preparations have been made of a variety of veins. The most interesting feature of these investigations is the sometimes striking difference in response of the veins *in vitro* to the same drug (Table I). The responses seem to depend upon the location from which the vein is taken. It should be noted that the preparations of rat veins behave like cardiac muscle, and both electrophysiological and histological evidence supports this (36). One additional feature of special interest is that rabbit external jugular vein seems to lack beta receptors.

UTERUS

The subject of adrenergic receptors in the myometrium was reviewed recently by Miller (38) so will not be considered in any detail here. Propranolol administration blocked the inhibitory effects of epinephrine (39) or isoproterenol (40) on the human uterus *in vivo* indicating the presence of beta inhibitory receptors. While this interpretation is reasonable, the type of antagonism is unknown and the effect of propranolol alone should be

studied in more detail. An apparent increase in uterine contractility of human subjects at term was noted during the infusion of propranolol (41).

The isolated human fallopian tube contracting in response to perivascular nerve stimulation was relaxed in the presence of phenoxybenzamine (42) but the relaxation was blocked by propranolol. This study suggests that tubal spasm might be relieved by alpha receptor blocking agents.

An isolated preparation of the guinea-pig, uterine horn with (ovarian) nerves attached has been described (43). Nerve stimulation produced motor responses during diestrus, and relaxation or irregular contractions during estrus. Norepinephrine produced similar effects during the estrous cycle, in this species.

An interesting hypothesis for the mechanism of the inhibitory effect of isoproterenol on the rat uterus was advanced by Schild (44). He postulated that isoproterenol activates a calcium-accumulating mechanism which lowers sarcoplasmic free calcium and leads to relaxation of the muscle. Relaxation of the rat uterus depolarized by K^+ occurred independently of the external calcium concentration. This experiment does not rule out the possibility that isoproterenol produces an active extrusion of calcium which might operate against a high concentration gradient.

Several interesting and important studies were made on antagonists of oxytocin. Sodium thioglycollate and 2-O-methyltyrosine-oxytocin antagonized oxytocin-induced contractions of the rat uterus but not those due to acetylcholine or bradykinin (45). These antagonists did not alter the resting potential, and their mechanism may involve a stabilization of the membrane of the myometrial cell. The studies of Krečí, Poláček & Rudinger (46) indicate that 2-O-methyltyrosine-oxytocin is best regarded as a partial agonist. The latter investigators attempted to define the conditions for agonism and antagonism. An oxytocin-like effect was produced more frequently on uteri from estrogen-treated rats and was dependent on the calcium and magnesium ion concentrations in the medium.

Chan, Fear & du Vigneaud (47) described two potent oxytocin inhibitors. These analogues of oxytocin, 1-L-penicillamine-oxytocin and 1-deaminopenicillamine-oxytocin, did not produce oxytocin-like effects. They competitively and reversibly antagonized the uterotonic response to oxytocin and related analogues on the rat uterus *in vitro* and *in vivo*; the responses to bradykinin and angiotensin were unaffected. Several other compounds were found to antagonize oxytocin but not to stimulate the rat uterus. These include N-acetyl-O-acetyloxytocin (48) and two acyclic synthetic oxytocin intermediates, the S-benzyltetrapeptide and S-benzyloctapeptide (49). These new oxytocin antagonists should be valuable aids in defining more completely the functions of oxytocin during normal and abnormal labor.

The pharmacologic significance of various functional groups of oxytocin for oxytocic activity was investigated by Chan & Kelley (50). The phenolic hydroxyl group and the carboxamide groups (positions 5 and 9) appeared to influence both intrinsic activity and affinity; the carboxamide group at position 4 was associated with drug affinity.

The sensitivity of the rat uterus to oxytocin *in vitro* is known to be affected by the magnesium concentration in the medium. Sensitivity to oxytocin was found to increase with increasing concentrations of magnesium up to a point, after which it declined (46). The influence of magnesium on the sensitivity of the rat uterus to oxytocin and related analogues varied with the oxytocic potency of the peptide (50, 51). Analogues which were relatively weak were potentiated to a much greater extent than those with higher activities. Potentiation was also noted on uteri depolarized by potassium, indicating that this effect of magnesium was not due to a propagated component of the response (51). The use of magnesium-free media for testing oxytocin analogues could lead to different relative potencies when assays *in vitro* are compared to those *in vivo* (50).

The mechanism of the contractile effect of prostaglandin-E_1 on the isolated rat uterus was investigated by Paton & Daniel (52). No evidence could be obtained for a mechanism involving acetylcholine or 5-hydroxytryptamine release. Low temperatures (17–24° C) were used to reduce spontaneous contractions in these experiments. The responses to both acetylcholine and prostaglandin-E_1 were greater in a magnesium-free medium. In the absence of calcium, acetylcholine but not prostaglandin produced contractions. The rapid disappearance of the responsiveness in a calcium-free medium, and recovery after exposure to calcium, suggests that contraction following prostaglandin-E_1 is due to a release of loosely bound calcium.

Unlike uteri from the rat and guinea pig, the human uterus is usually relaxed by prostaglandins in the E series. The inhibitory effect of prostaglandin-E_1 on the isolated human uterus was not modified by propranolol or dihydroergotamine, indicating that the response is not mediated by adrenergic receptors (53). The relative inhibitory potencies of a series of prostaglandins present in human semen were estimated on the isolated human uterus (54). The response to a mixture of several prostaglandins indicated that the effects of the individual compounds were additive.

A strongly basic polypeptide with properties similar to relaxin was extracted from ovaries of pregnant sows by Griss *et al.* (55). After purification by several procedures, a chromatographically and electrophoretically pure material was obtained This factor inhibited uterine motility of the isolated rat and guinea-pig uterus but not human uteri. In earlier studies, it was found that the isolated human uterus was not inhibited by relaxin-containing ovarian extracts (56). Evidence was obtained in subsequent studies that relaxin produced inhibition indirectly by causing a release of endogenous catecholamines from the uterus, the most important of which was epinephrine (57, 58). The threshold dose of relaxin was inversely proportional to the epinephrine content of the rat uterus during the estrous cycle and after various drug treatments (58). The inhibitory effect of cycloheximide may be due to a liberation of catecholamines from the rat uterus (59).

The initial rate of spontaneous uterine contractions varied indirectly with the endogenous epinephrine content of the rat uterus during the es-

trous cycle, after various estrogens or other drug treatments (58, 60). In the human, the epinephrine content of the uterus was highest during the proliferative phase of the menstrual cycle, declined during the secretory phase (61), and was not detected in uteri from menstruant women (62). Changes were also noted in the catecholamine content at placental and non-placental sites (61, 60). Factors affecting the active uptake and release of catecholamines by the rat uterus were investigated by Green & Miller (63, 64). Evidence was presented (65) for the presence in the female reproductive tract of two types of adrenergic neurons with different morphological and functional characteristics.

The exchange of calcium in rat uteri was studied by Feinstein (66). The washout of radioactive calcium in calcium-free solutions could be resolved into at least three exponential phases. Tetracaine decreased the efflux of radioactive calcium brought about by the addition of cold calcium during the slowest exponential phase of washout. Procaine and tetracaine appeared to block competitively the uterine contractions produced by calcium on uteri depolarized by potassium. The inhibition of spontaneous uterine contractions produced by tetracaine was antagonized by calcium. These findings suggest that local anesthetics can interfere with the exchange of calcium by the rat uterus.

An interesting correlation was reported to exist between the activity ratios, relative to histamine, of a series of beta-substituted ethylamines for their capacities to produce gastric acid secretion and inhibition of the rat uterus (67). These effects of histamine are not antagonized by antihistamines. It might be possible to use the uterine preparation to evaluate substances which antagonize the effect of histamine on gastric secretion.

Trachea and Lung

The effects of prostaglandin-F_{2a} and -E_2, which have been found in human lung, have recently been studied on isolated human bronchus (68). Since prostaglandin-F_{2a} caused contraction while E_2 (and E_1) relaxed the muscle, the authors suggest that a functional antagonistic relationship seems possible.

Other studies of the actions of the prostaglandins on the lung and tracheal muscle have been thoroughly discussed in a recent review (33) and will not be dealt with further here.

The actions of halothane in increasing compliance and decreasing pulmonary resistance may (69) be mediated through stimulation of beta receptors. Explanations based on the release of catecholamines or an axon reflex were eliminated by suitable procedures and also because the responses were specifically blocked by MJ 1999 or pronethalol. The responses of the uterus and heart to halothane could also be blocked by MJ 1999 [unpublished observations quoted in (69)].

Roberts (70) has presented persuasive evidence for the artifactual formation of an isoproterenol-like substance from epinephrine under conditions

in which such a substance has been "isolated" from mammalian tissue. The significance, therefore, of the isolation of an isoproterenol-like catecholamine after stimulation of the bronchial sympathetic nerves (71) is open to serious question.

Considerable effort has been devoted recently to the elucidation of adrenergic receptors and mechanisms present in trachea (72–75) and lungs (76). All these workers have agreed upon the existence of beta inhibitory receptors in isolated tracheal smooth muscle based on potency order: isoproterenol > epinephrine > norepinephrine > phenylephrine [except in cat trachea (73)] and the blockade of catecholamine-induced relaxation of a variety of beta receptor blocking agents.

Potentiation of the inhibitory effects of the catecholamines by certain alpha adrenergic blockers has also been consistently observed. Such effects have been variously explained by supposing that beta haloalkylamines (73, 74) and tolazoline (74) block nonspecific receptors or alpha receptors in the trachea, or inhibit the uptake of catecholamines (73, 72). It seems unlikely that a specific blockade of silent (73) or functional (74) alpha receptors could alone account for the potentiation. The concentrations at which potentiation is observed are usually many times those necessary for the blockade of alpha receptors in other tissues, but within the range shown by Iversen to inhibit $Uptake_1$ and $Uptake_2$ (77). Foster (72), in his extensive study of the effects of a variety of alpha adrenergic blocking agents, found marked differences in the ability of these agents to cause potentiation: piperoxan and dihydroergotamine caused no potentiation, thymoxamine and hydergine caused a small potentiation of norepinephrine (but not epinephrine or isoproterenol), phentolamine caused potentiation of norepinephrine and isoproterenol, and phenoxybenzamine caused a slowly developing and large potentiation of all three catecholamines. The differences observed in the actions of these agents may ultimately be found to be due more to the peculiarities of the individual drugs than to differing effects on uptake.

The study of Takagi et al. (74) stands in contrast to other *in vitro* studies in one important respect. He found, after blockage of inhibitory responses of the trachea by either of two beta blocking agents, that epinephrine, norepinephrine, and phenylephrine (but not isoproterenol) caused contraction of the guinea-pig trachea which was blocked by dibenamine or tolazoline. The order of potency for producing contraction was as above, and opposite to the broncho-constrictor potency of these amines found in the guinea pig *in vivo* (76). In a recent study, Chahl & O'Donnell (78) observed increases in tone of tracheal muscle which had been exposed to norepinephrine or epinephrine after propranolol. They also suggest that a few alpha excitatory receptors may exist in trachea. Variations in technique do not seem to account for such qualitative differences but strain differences in guinea pigs might be responsible.

The recent *in vivo* study by Lynn James (76) using the Konzett-Rössler method presented evidence for the existence of both alpha stimulatory and

alpha inhibitory receptors in guinea pig lungs. Epinephrine, norepinephrine, and phenylephrine produced bronchoconstriction; isoproterenol did not. The order of potency in causing this effect was characteristic of alpha receptors, but alpha blocking agents were not examined for their ability to inhibit this response. The presence of alpha stimulatory receptors has been reported in isolated guinea pig lung (79) and in dog lung *in vivo* (80). The existence of alpha inhibitory receptors was postulated because only tolazoline antagonized phenylephrine-induced bronchodilation, while pronethalol potentiated it. It is probably superfluous to state that the reactions of "lungs" *in vivo* to drugs may not be perfectly related to receptor mechanisms of trachea elucidated *in vitro*.

Foster has presented impressive quantitative evidence for a causal relationship between the inhibition of uptake by various drugs and the potentiation of norepinephrine in the guinea pig tracheal preparation (81). The amount of potentiation of norepinephrine was found to be time dependent; cocaine, metanephrine, and cooling potentiated rapidly while desipramine, guanethidine, and phenoxybenzamine acted more slowly. The relative potencies and concentrations of the drugs which inhibited norepinephrine or epinephrine uptake *in vivo* correlated amazingly well with the relative potencies and concentrations of drugs which potentiated norepinephrine. In addition, the ability of the drugs to potentiate the response to isoproterenol relative to that of norepinephrine corresponded with the demonstrated ability of these drugs to inhibit $Uptake_2$ with respect to $Uptake_1$ [see (81) for a summary of references]. An independent study by Chahl & O'Donnell (78) of the interactions of cocaine and propranolol with three catecholamines on a similar tracheal preparation produced similar results. Cocaine potentiated all three catecholamines in the order norepinephrine $>$ epinephrine $>>$ isoproterenol, consistent with their affinity for $Uptake_1$ (77). After competitive inhibition of the responses by propranolol, cocaine had little effect on the catecholamines, significantly potentiating only epinephrine. This is consistent with the existence of a second uptake mechanism, active at high amine concentrations (as after propranolol), insensitive to cocaine, and having greatest affinity for epinephrine. This resembles closely the $Uptake_2$ process in the rat heart, the relative affinities being epinephrine $>$ isoproterenol $>>$ norepinephrine (82).

Intestine

Paton & Zar (83) prepared strips of longitudinal muscle from the guinea-pig ileum retaining or free from Auerbach's plexus. In the absence of the plexus, the longitudinal muscle failed to respond to electrical stimulation, nicotine, dimethylphenylpiperazinium iodide, or physostigmine. Acetylcholine was not detected in the strips nor in the medium after stimulation. In contrast, strips retaining Auerbach's plexus responded to electrical stimulation or ganglionic stimulants, and acetylcholine was found in the strip and in the medium after field stimulation. The longitudinal smooth muscle

does not appear to be the source of acetylcholine and it is probably not released from smooth muscle cells as a local transmitter. By using innervated and denervated strips, the extent to which a drug response was due to an effect on muscle cells or the nerve plexus was estimated (Table II). In a similar study, tetrodotoxin was employed by Gershon (84) on several gastrointestinal preparations to differentiate drug responses due to direct muscle stimulation from those resulting from nerve stimulation. Conduction of nerve action potentials is blocked by tetrodotoxin but the electrical activity of smooth muscle is not abolished. The effect is similar to denervation, the action of drugs which act wholly or in part by nerve stimulation would be

TABLE II

SITE OF ACTION OF VARIOUS DRUGS ON THE GUINEA-PIG ILEUM

Nerve	Muscle	Nerve and Muscle
Nicotine (83, 84)	Acetylcholine (83, 84)	Angiotensin (83, 84)
Dimethylphenyl-piperazinium iodide (83, 84)	Bradykinin (84)	5-Hydroxytryptamine (83, 84)
	Histamine (83, 84)	Barium chloride (83, 84)
	Oxytocin (83)	Potassium chloride (83, 84)
	Arecoline (83)	
	Muscarine (83)	
	Tremorine (83)	

abolished or reduced in the presence of tetrodotoxin. Gershon cautioned, however, that residual responses to a drug after tetrodotoxin could be due to transmitter release not requiring a conducted action potential. An anticholinesterase and atropine were used in a search for this mechanism but none of the agents which he tested (Table II) acted in this manner. Tetrodotoxin (85) prevented the contractile response produced by transmural stimulation or nicotine, reduced that produced by 5-hydroxytryptamine, and had no effect on the actions of acetylcholine and histamine. These findings are consistent with those summarized in Table II.

The contractions of longitudinal and circular muscle of the isolated human ileum produced by 5-hydroxytryptamine could not be antagonized by atropine, procaine, or hexamethonium (86). No evidence was obtained that stimulation was mediated by the intrinsic nerves and it is likely that in the human, 5-hydroxytryptamine can act directly on muscle cells of the terminal ileum.

The relative potencies of a series of narcotic analgesics were similar for analgesia (mouse hot-plate) and inhibition of the isolated guinea-pig ileum (87). There was good agreement between the inhibitory effects of morphine on acetylcholine release and contractions produced by electrical stimulation of the ileum. The depressant effect of morphine on contractions evoked by coaxial stimulation was antagonized by various narcotic antagonists as well

as by small concentrations of morphine itself (88). A correlation was also noted between the concentrations of the convulsant drugs strychnine, brucine, picrotoxin and nikethamide required to inhibit contractions produced by coaxial stimulation and their convulsant doses (89). These drugs, like morphine, appeared to act by preventing the release of acetylcholine. However, rather large concentrations ($10^{-2} - 10^{-4}$ *M*) were required for inhibition. These correlations may reflect more the capacity of these agents to gain access to their sites of action than their mechanisms of action.

The release of acetylcholine from the isolated guinea-pig ileum in the presence of various concentrations of 5-hydroxytryptamine and dimethylphenylpiperazinium was investigated by Brownlee & Johnson (90). Both substances released acetylcholine but, per molecule, 5-hydroxytryptamine appeared to release relatively more. The contractions produced by either substance were related to the acetylcholine released. It is known, however, that some of the effect of 5-hydroxytryptamine is due to its direct action on smooth muscle (Table II) making it difficult to relate the contractile response to only the acetylcholine which is released.

Peristaltic contractions produced by coaxial electrical stimulation or by increasing intraluminal pressure of the guinea-pig ileum were depressed by hemicholinium or by triethylcholine (91). The effects of the latter two agents were reversed by choline. In a similar study (92), elevations of intraluminal pressures produced proportionate increases in acetylcholine release. Both morphine and triethylcholine reduced the release of acetylcholine.

A relaxation of the rat ileum was produced by acetylcholine in the presence of scopolamine (93). This relaxation was blocked by reserpine pretreatment, alpha and beta receptor blockade, or adrenergic blocking agents, but not by ganglionic blocking agents. These results indicate that this effect of acetylcholine was mediated by a sympathetic mechanism. Transmural stimulation of the rabbit intestine usually produced an inhibition of spontaneous contractions followed by stimulation and in turn by a period of inhibition (94). The inhibition was most prominent at lower temperatures and was not blocked by guanethidine or mixtures of alpha and beta receptor blocking drugs. The results suggested that the inhibition was due to activation of nonadrenergic inhibitory neurons.

The uncharged carbon analogue of acetylcholine, acetylcarbocholine (3,3-dimethylbutyl acetate), produced a contraction of the guinea-pig ileum which was blocked by atropine (95). When considered as an agonist it appeared to have an affinity much less than acetylcholine. A more detailed study of the mechanism of action of acetylcarbocholine (96) indicated that it acts indirectly by releasing acetylcholine, probably from nerve endings. Like phenyl acetate (97), its action was blocked by hemicholinium or procaine, but, unlike nicotine, activity was not prevented by hexamethonium. It also appears that a number of acetic acid esters of aliphatic alcohols act by liberating acetylcholine from the guinea-pig ileum (98). These rather sim-

ple esters could be useful for investigating mechanisms for acetylcholine release. The effects of acetylcarbocholine and probably of many of the other esters are complicated, since they also possess weak atropine- and papaverine-like actions (96).

The sites of action of several cholinesters and alkyltrimethylammonium compounds on the intestine of the rat were investigated by Takagi, Takayanagi & Maezima (99). The responses to apparent partial agonists such as butyrylcholine and ethyltrimethyl-ammonium iodide were reduced by cooling (13° C), anoxia, and cocaine. On the guinea-pig ileum, the effects of cholinesters which behaved as partial agonists were also reduced by these treatments and, in addition, by hexamethonium and tetrodotoxin (100). In these two studies the response to a complete agonist such as acetylcholine appeared to be little affected. These results suggest that the partial agonists act indirectly by releasing acetylcholine, possibly by ganglionic stimulation, and not by interacting directly with smooth muscle receptors. At low temperatures these substances could be shown to antagonize acetylcholine and would be expected to block, at least in part, any acetylcholine which they release. These interesting studies by Takagi, Takayanagi & Maezima raise important questions on the recognition of partial agonists and challenge the concept of intrinsic activity, since compounds which appeared to be partial agonists in these investigations simply acted by a different mechanism of action than the complete agonists.

The protonated forms of arecoline (95, 101, 102) and related derivatives appear to be the active species in this group of tertiary parasympathomimetic drugs. With compounds of this type, the degree of protonation must be taken into consideration in the calculation of pD_2 values (102). The apparent intrinsic activities of these tertiary amines increased with increasing pH values which may relate to their accessibility to receptor sites.

High concentrations of sodium ascorbate were found to have a direct stimulant action on the guinea-pig ileum (103). Segments of the ileum from guinea pigs fed a diet deficient in ascorbic acid were less sensitive to acetylcholine compared to segments from control animals. The sensitivity was restored by adding ascorbic acid to the diet or to the isolated tissue bath.

Vas Deferens

Much of the recent work involving the vas deferens or hypogastric-vas deferens interrelationships has been concerned with gathering evidence for or against the hypothesis of a cholinergic link in adrenergic transmission.

Since Ferry has recently summarized the evidence for and against such an hypothesis in his excellent discussion (104) only brief mention will be made here of this aspect of the pharmacology of the vas deferens. Bell (105), Birmingham (106), and Bell & McLean (107) have presented electrophysiological, pharmacological, and histological evidence, respectively, for the existence of separate postganglionic adrenergic (major) and cholinergic (minor) innervation of the guinea-pig vas deferens. In particular, it

was interesting to note the absence of catecholamine fluorescence from acetylcholinesterase-containing neurons and vice-versa in the hypogastric ganglion of the guinea pig (107). Graham, Al Katib & Sprigg (108) present similar evidence for the rat, the cholinergic element being quantitatively more pronounced than in the guinea pig.

An interesting report has appeared (109) relative to the adrenergic receptor population of the vas deferens. Isoproterenol and epinephrine were both found to reduce the response of the guinea-pig vas deferens to nerve stimulation in low concentrations and to increase it at higher concentrations. This effect of epinephrine was potentiated by phentolamine and the inhibitory effects of both amines were blocked by dichloroisoproterenol and pronethalol. Thus, sympathetic beta receptors have been demonstrated in the vas deferens.

One of the most intriguing findings in recent years is that 6-hydroxydopamine (2, 4, 5-trihydroxyphenylethylamine) causes degeneration of adrenergic nerves which leads to a "chemical sympathectomy". Tranzer & Thoenen recently (110) demonstrated by electron microscopy degeneration (at 3 days) and disappearance (at 2 weeks) of adrenergic nerve terminals in vas deferens, iris, spleen capsule, and right auricle of the cat. The selective degeneration of adrenergic nerves occurs only in the distal part of the neuron and reinnervation is complete within 3 to 4 months. A study of adrenergic neuron fluorescence in various tissues of the mouse (111) revealed rapid disappearance of specific fluorescence in iris and heart but very little effect on the fluorescence of the superior cervical ganglion and vas deferens. The lack of effect on the ganglion is in accord with the known insensitivity of cell bodies (compared with nerve terminals) to this agent, but the lack of effect on the vas deferens is puzzling, especially since 6-hydroxydopamine has an excellent effect in the cat. Desmethylimipramine prevents the action of 6-hydroxydopamine while reserpine potentiates its action on other tissues in the mouse indicating that uptake into (or onto) the neuron but not the storage granule is necessary for its action. It is difficult to understand why the neurons of the vas deferens should be different from those in other tissues in their ability to take up amines. The contractions of the nictitating membrane of the dog in response to preganglionic stimulation were likewise little affected by pretreament with this drug (112).

An enlightening paper has been published by Barnett, Greenhouse & Taber (113) on the increase in the maximum response to cumulative doses of norepinephrine produced by amitriptyline, imipramine, desmethylimipramine, dexchlorpheniramine, phentolamine, and cocaine. Although all these drugs are known to inhibit uptake, this cannot account for their ability to increase the maximum response to norepinephrine since (*a*) their potency in increasing the maximum response does not correlate well with their relative ability to block uptake; and (*b*) potentiation and increased maximum response can be dissociated by washing the preparation. One ex-

planation for these results would be the prevention of the desensitization process of autoinhibition. Two observations make this likely: (*a*) the increase in maximum response produced by all agents was about equal and equivalent to the decrement in response caused by autoinhibition (evaluated separately); and (*b*) the increase in maximum response was independent of whether the agent caused potentiation or blockade of norepinephrine responses. Autoinhibition has been shown to occur also in rabbit aorta (1, 3) and cat spleen (114). If such an action of cocaine can take place in these organs, which seems likely, then the puzzling increase in maximum response to norepinephrine after cocaine, observed by Bevan & Verity (12, aorta) and Reiffenstein (114, spleen), can be explained without invoking a direct sensitizing action of cocaine or a "deformation" of receptors.

Spleen

Discussion of recent publications on the spleen will be restricted to a consideration of drug interactions with isolated splenic strips, One exception will be made to point out the report by Hertting & Suko (115) which emphasizes the importance of changes in perfusion flow rate of the isolated spleen in the analysis of drug effects.

Ignarro & Titus (116) have investigated the adrenergic receptors of the mouse spleen with interesting results. The presence of alpha stimulatory and beta inhibitory receptors were unequivocally demonstrated by affinity (pD_2) ranking and blockade by specific blocking agents. Epinephrine and isoproterenol reversal could be demonstrated after phenoxybenzamine and rereversal after MJ 1999. It is interesting that their illustrations of the antagonism of norepinephrine, epinephrine, and isoproterenol by phenoxybenzamine demonstrate that little receptor reserve exists for these agents in the mouse spleen, (i.e. the maximum response decreases before appreciable shifts of the curve occur).

Other workers (117), examining the correspondence of catecholamine concentration-response curves in cat splenic strip with theoretical curves based on the mass action laws, have noted departures from theory with *dl*-isoproterenol. If the concentration-response curves for isoproterenol were "corrected" by a computer program for "threshold", the curves then corresponded to theoretical ones as did those for *l*-epinephrine and *l*-norepinephrine. The slope was also now parallel to the other two agonists. The authors acknowledge the possibility of the influence of the *d*-isomer, a known alpha blocking agent (118), on the concentration-response curve, but consider it unlikely to be a major factor. Thus, there may exist a threshold for *dl*-isoproterenol in cat spleen, i.e., a certain number of receptors must be occupied by this agent before a contraction of the tissue is obtained.

Two papers have appeared which consider the mechanism by which cocaine (119, 114) and TK 174 (3,3-di(*p*-aminophenyl) propylamine) (119), potentiate the effects of catecholamines on the isolated cat spleen. Cocaine and TK 174 both potentiate the actions of norepinephrine and to a lesser ex-

tent isoproterenol on the spleen. Since the effects of isoproterenol were potentiated, the authors concluded that inhibition of uptake by cocaine could not wholly account for its sensitizing action. It is interesting that cocaine will not potentiate the beta inhibitory effects of isoproterenol but only the alpha stimulatory effects in tissues which lack beta receptors: central artery of the rabbit ear (11) and cat spleen (119, 116). Reiffenstein (114) reported that cocaine did not potentiate the contraction rate of the spleen to norepinephrine but that it did, as in Bevan & Verity's study (12), potentiate the final contraction height. To explain this, he also suggested that cocaine deformed adrenergic receptors to allow an increased utilization. An excellent explanation for this effect has already been discussed under VAS DEFERENS (*quod vide*) so that it is not necessary to postulate a deformation of the receptor to account for this observation. Green & Fleming have also concluded that cocaine does not deform receptors (120).

NICTITATING MEMBRANE

Recent studies utilizing the nictitating membrane of the cat have focused upon the analysis, at the receptor level, of changes produced by various sensitizing procedures. Green & Fleming have used an ingenious approach for the determination of *in vivo* "pA_2" and "pD_2'" values in their study (120). They determined the effect of four procedures (cocaine, chronic denervation, chronic decentralization, and reserpine pretreatment) on the pA_2 values for norepinephrine/phentolamine and the pD_2' values for norepinephrine/phenoxybenzamine. None of the four treatments, all of which produced supersensitivity, caused any change in the pA_2 values. This indicated that deformation of adrenergic receptors did not occur, since affinity for the receptors did not change. The pD_2' values after denervation, decentralization, and reserpine treatment were significantly lower than control indicating either an increase in the number of receptors or that the relationship between occupation and response had changed, both of which would indicate that a postsynaptic change had occurred. Also, all three treatments produced the same magnitude of change, possibly indicating a common mechanism in the development of supersensitivity. Since neither pA_2 nor pD_2' changed after cocaine administration, it is highly unlikely that postsynaptic changes are involved in its mechanism of action.

Varma's (121) hypothesis that a qualitative change in alpha receptors is produced by denervation was based in part on the relative ineffectiveness of phentolamine in inhibiting norepinephrine responses in the denervated nictitating membrane. Green & Fleming (120) have suggested that the presence of "tone" in his preparations has affected his results.

Additional evidence for the nonspecificity of supersensitivity following denervation or reserpine pretreatment has been presented by Schmidt & Fleming (122) and Hudgins & Fleming (123). The first authors found that supersensitivity of the nictitating membrane develops not only to catecholamines, but also to acetylcholine, serotonin, tryptamine, and barium. Hudgins

& Fleming found that the development of nonspecific supersensitivity was not restricted to the nictitating membrane but extended also to aortic strips. This is consistent with a change in the physiology of the smooth muscle cells but not of the receptors.

Tye, Patil & LaPidus have shown the sensitization to cocaine in the nictitating membrane to be sterospecific for D (−)- over L (+)-isomers of some sympathomimetics, thus providing additional evidence for the presynaptic action of cocaine (124).

Drug Receptors in Smooth Muscle

Considerable effort has been directed to the study of drug receptors in smooth muscle. Receptors have been characterized by the use of selected agonists and antagonists, and various constants for drug-receptor interactions have been obtained by the mathematical analysis of dose-response curves. The literature and status of this subject have been considered ably by several authors (125–129) and will not be dealt with here. Rather, attention will be directed to the recent attempts to label receptors and to efforts made to characterize further the conditions for receptor activation.

Phenoxybenzamine and related beta haloalkylamines are thought to produce a blockade of the alpha adrenergic receptor by combining with it covalently. The blocking effect of these alkylating agents can be prevented by the presence of appropriate concentrations of an alpha receptor agonist or antagonist. These substances probably prevent receptor alkylation and thereby produce a receptor protection which is specific for a particular class of drugs. It is reasonable to expect that less alkylation of a tissue might occur if receptors were protected by a particular agonist or antagonist. The decrease in alkylation might therefore relate to the number of sites protected and, if only the receptors were protected, provide a means to estimate the number of receptors and study their properties. Several laboratories have investigated these possibilities by the use of labeled alkylating antagonists.

A difference in the tissue radioactivity after labeled dibenamine was noted when rabbit aortic strips were protected with epinephrine (130, 131). No evidence could be obtained that the receptor for acetylcholine is cephalin (131, 132) as postulated by the earlier investigators (130) using a similar procedure.

Labeled phenoxybenzamine was employed to investigate the alpha adrenergic receptor in the seminal vesicle of the rat (133). Phentolamine was capable of protecting nearly all of the receptors for norepinephrine as judged from dose-response curves. There was less radioactivity in the nonlipid residues of tissues that were protected with phentolamine. From the difference in radioactivity of protected and nonprotected preparations it was estimated that there may be as many as 55,000 alpha receptors per smooth muscle cell in the seminal vesicle (Table III). No differences were found in the lipid-containing extracts, indicating that the receptor is probably present

TABLE III
QUANTITATIVE ESTIMATES OF DRUG RECEPTORS[a]

Type	Tissue	Number per cell	Moles/g (wet weight)	Percentage of Cell Surface Covered	Reference
Alpha Adrenergic	Seminal Vesicle (rat)	55,000	2.8×10^{-11}	.004	133
Alpha Adrenergic	Aorta (rabbit)	—	58×10^{-11}[b]	—	135
Beta Adrenergic	Atria (guinea pig)	100,000	1.8×10^{-11}	.004	139
Muscarinic	Intestine (guinea pig)	16,000	2.0×10^{-11}[c]	.002	140

[a] Maximal values.
[b] Calculated from dry weight assuming 70 per cent water.
[c] See footnote 4 in text.

in the nonlipid residue. Norepinephrine produced a relatively small degree of protection while cocaine and a beta receptor blocking agent produced none. The latter two agents did not significantly reduce the binding of phenoxybenzamine, and norepinephrine produced only a small effect. No significant receptor reserve for norepinephrine could be shown to exist in the seminal vesicle.

A variety of amines including methamphetamine, amphetamine, tyramine, benzylamine, and *d*-norepinephrine were reported to decrease the uptake of the labeled alkylating agent, SY-28 (N-(2-bromethyl)-N-ethyl-N-naphthylmethylamine), by the rabbit vas deferens (134). The authors suggest that the decreased uptake of this blocking agent may be due to some protection of uptake and storage sites for norepinephrine. However, it may be noted that many of these agents are also known to release endogenous norepinephrine at the receptor site which could produce a receptor protection. A short-acting "irreversible" alpha receptor antagonist, DBP (N,N-dimethyl-2-bromo-2-phenethylamine), produced little effect on the binding of SY 28 *in vitro* (rabbit aortic strip) but appeared to do so on several tissues *in vivo*. However, SY-28 appeared to decrease the binding of DBP *in vitro* (135). The number of alpha receptors in the rabbit aorta (Table III) was calculated by following the loss of radioactivity from strips treated with labeled DBP. This estimate would represent a maximum figure since loss could occur from receptor and nonreceptor sites.

The muscarinic receptors for acetylcholine were blocked by benzilylcholine mustard (BCM; N-2-chloroethyl-N-methyl-2-aminoethyl benzilate) and evidence was obtained for receptor alkylation of long duration (136). The

uptake of labeled BCM by longitudinal muscle of the guinea-pig intestine was decreased by atropine and related agents but not by cocaine, tubocurarine, pentolinium, mepyramine, or physostigmine (137).

Further study is necessary to evaluate the use of labeled alkylating antagonists for investigating receptors in an experimental design employing receptor protection. It is clear from the present studies that nonreceptor alkylation can occur. There are many potential sites of alkylation since complete tissue saturation has not been achieved (132, 134, 138). Thus, it is possible that alkylation of nonreceptor sites could occur even when receptors are protected from alkylation, thereby making it difficult to detect differences in tissue radioactivity in protected and nonprotected preparations. By fractionation or extraction of cellular material it may be possible to estimate the contribution made by nonreceptor alkylation. The choice of the protecting agent is also a factor since it is possible that the substance could prevent alkylation of the receptor as well as certain nonreceptor sites. Table III shows estimates of the number of receptors of various types in several tissues.[4] These represent maximal values and the actual members could be less. Without considering the different procedures employed and the assumptions made, with one possible exception, the similarities of these estimates are striking and not unlike Clark's (141) earlier values. Although they all represent receptors for neurotransmitters in the autonomic nervous system, there is little a priori reason to believe that they should be so similar.

Trypsin was reported by Graham & Katib (142) to reverse the adrenergic blockade produced by several beta haloalkylamines. These investigators speculated that this enzyme could catalyze the breakage of an ester linkage between the alkylating agent and the carboxyl group of arginine or lysine. The location of the receptor was regarded to be on the cell membrane; the receptor appears to consist of a peptide containing arginine or lysine with a free carboxyl group. Other investigators (134) concluded that the recovery of response after trypsin was unrelated to alpha receptor regeneration.

The protection of alpha receptors from phenoxybenzamine blockade *in vivo* was produced by a number of competitive adrenergic blocking agents including phentolamine, trifluoperazine, dextroisoproterenol, and dextrohyoscyamine (143). Protection of receptors from blockade was indicated by observing the lethal effect of epinephrine in rats. A correlation was obtained between alpha receptor protection and adrenergic blocking activity.

The relative potencies of a number of derivatives of norepinephrine with substitutions on the alpha carbon or nitrogen center were determined on several isolated tissue preparations (144). Two rankings were obtained and considered to be evidence for two types of beta adrenergic receptors, $beta_1$

[4] Some confusion exists in the literature as to Paton & Rang's estimate. They (140) clearly distinguish their estimate of *uptake* capacity (180 pM/g) from *receptor* capacity (20 pM/g).

and beta$_2$. The heart, adipose tissue, and small intestine appeared to constitute one group (beta$_1$) while the rat uterus, diaphragm, bronchioles, and vascular bed were included in a second group (beta$_2$). Similarly, selective differences in beta receptor blockade have been observed in the substituted methoxamine series of antagonists where the compounds also possess an alpha methyl group, [e.g., (145, 146)]. Except for one compound, all would seem to be selective beta$_2$ blockers, although the author would prefer to view the compounds as being selective, rather than the receptors. The studies of Palm, Langeneckert & Holtz (147) indicate that there are differences between the beta receptors in the heart and vasculature. Also, the beta receptors in the rat uterus appeared to possess characteristics which differ from those in the heart (38).

The alpha receptor activity of the rabbit intestine is lost or impaired after storage at 6 to 8° C for 24 to 72 hr (148, 149). The inhibitory effect of methoxamine or phenylephrine is reduced or absent but epinephrine, norepinephrine, and isoproterenol are effective after storage, indicating the presence of beta receptor activity (148). The loss of responsiveness to methoxamine does not appear to be due to hypoxia, catecholamine depletion, loss of nerve function, or loss of a stable tissue constituent (149). It would be interesting to investigate the effect of such storage on the alpha receptor activity in other tissues.

A Note on Methods

A useful manual for pharmacological experiments on isolated tissue preparations has been prepared by the staff of the Department of Pharmacology at the University of Edinburgh (150). Current methods for studying uterine drugs have been summarized conveniently in a review by Berde & Saameli (151).

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