In Vitro Action of Mescaline

Possible Mode of Action

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Mescaline (TMPEA) elicited a motor response (dependent upon segment and concentration) from isolated guinea pig, rat, and cat ileum. It did not alter inotropic or chronotropic activity of isolated mammalian atria. Estrogen dominated uteri responded similarly. TMPEA antagonized serotonin-induced motor responses of rat and guinea pig uteri. High concentrations of ganglionic blocking agents potentiated TMPEA motor effects. Evidence suggests that TMPEA does not act *via* a cholinergic mechanism, but rather through catecholamine mechanisms. The end result varies in definite situations. The primary site of action of TMPEA appears to be at the α-adrenergic receptor site.

THE LITERATURE contains few reports concerning the effects of mescaline (trimethoxyphenylethylamine: TMPEA) on peripheral systems. This is especially true of investigations involving *in vitro* systems. Most of the available data seem to have been compiled from studies in which TMPEA was compared to other psychotomimetic compounds, rather than from research into the effects of TMPEA *per se.*

It was observed by Grace (1) that duodenal preparations from the cat and rabbit, as well as uteri obtained from the rat, rabbit, and guinea pig, did not respond to TMPEA. On the other hand, that investigator reported that TMPEA produced powerful contractions in the rabbit intestine *in situ* and that this effect could be abolished by intravenously administered atropine. Subsequently, Costa (2) demonstrated in *in vitro* studies that atropine did not block the effect of TMPEA on rat uteri. Costa (2) also reported that very low concentrations of lysergic acid diethylamide (LSD) and TMPEA synergized the effects of serotonin on uterine tissue from estrogen-primed rats.

There appears to be a consensus that TMPEA does not antagonize the response of rat uteri to serotonin. However, a number of investigators have reported the antiserotonin activity of LSD (2–5).

Serotonin produces contraction of umbilical vessels of human placenta, and TMPEA has been shown to cause constriction of the same preparation (4). The latter reported also that while LSD and tryptamine antagonized the effect of serotonin, TMPEA usually did not alter the response to either serotonin or epinephrine.

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In *in vivo* systems, Parker and Hildebrand (6) found TMPEA to cause a rapid rise in blood pressure when it was administered intravenously to anesthetized cats, and that this response could be blocked by dibenamine. Speck (7) reported similar vasoactive properties for TMPEA in the rat, but that investigator observed antagonism between TMPEA and epinephrine.

The structural similarity between TMPEA and epinephrine has been an important factor in the elaboration of theories which tend to explain psychotomimetic action as involving the catecholamines in some fashion. Nevertheless, studies of the interaction of TMPEA and the neurohormones are relatively rare. Thus, Giarman and Freedman (8) cited the work of Waser *et al.* (9) in stating that serotonin, norepinephrine, and histamine were affected similarly in blood and brain by both TMPEA and LSD. In view of reports of cross-tolerance between TMPEA and LSD in both humans and animals, these findings have been interpreted as suggesting a receptor site common to TMPEA and LSD.

Schopp and his co-workers (10) presented further evidence which demonstrates an interaction between TMPEA, epinephrine, and acetylcholine in peripheral nerve transmission.

In distribution studies of TMPEA in the dog, Cochin *et al.* (11) recovered that compound from ventricular tissue. This finding is especially interesting in light of reports that TMPEA has a variable effect on pulse and blood pressure (12, 13).

In view of the above findings, *in vitro* studies were undertaken to determine the effect of TMPEA on isolated structures from different species and to assess those effects by the use of agents which might aid in the elucidation of those activities. In addition, TMPEA was compared to drugs of known similar and dissimilar activities. In this way, it was expected that some knowledge of the mechanism of action would be

obtained, so far as peripheral effects were concerned.

EXPERIMENTAL

Various smooth muscle tissue from different species was used to observe the activity of TMPEA. Adult male and female guinea pigs weighing between 800 and 1000 Gm. were utilized. The animals were sacrificed by exsanguination. The selected tissue was removed (ileum, esophagus, aorta, trachea, duodenum, jejunum, and atria) and placed in a 20-ml. tissue bath containing Tyrode's solution.[1] When uterine tissue was used, De Jalon's solution[2] was employed as the bathing medium. The bath was aerated with a mixture of 5% CO_2 and 95% O_2 at a flow rate of 30 ml./min., for all tissues except the atria. In the latter case, flow rate was maintained at 60 ml./min.[3] The bath was thermostatically controlled at 37.5° (±0.5°). The tissue was connected to a light magnesium lever and the activity of the tissue was recorded on a slow-moving, smoked paper kymograph (0.791 or 5.18 cm./min.). Fasted and unfasted guinea pigs were used when uterine tissue was studied.

Guinea pig tracheal tissue was prepared by cutting the tissue into spirals. The responses to TMPEA and other compounds were recorded after a pilocarpine-induced spasm had reached a stable plateau (14), unless otherwise stated.

Uterine tissue from unfasted albino rats weighing between 200 and 250 Gm. (inbred strain, Food and Drug Research Laboratories, Maspeth, N. Y.) was challenged similarly to the tissue from guinea pigs. Tissue from rats previously mated and determined to be in estrus was used. It had been observed that tissue from these animals was able to withstand more challenges than virgin tissue. Determination of estrus was made according to Papanicolaou's method (15). The nictitating membrane, right atria, and ileum from fasted kittens (500–850 Gm.) were similarly challenged. Removal of these tissues was accomplished while the animal was anesthetized with sodium pentobarbital (35 mg./Kg.). Nictitating membrane and atrial tissue was allowed an adaptation period of 1 hr. prior to drug challenge. It was noted that atrial tissue required this period of time for stability. Challenges were performed at 15-min. intervals or when control activity was reestablished. Animals from which ileum or other intestinal tissue was removed had been fasted for approximately 16 hr. Some animals (guinea pigs in particular) were fasted for prolonged periods (36–48–60 hr.) in order to evaluate the effect of long periods of fast on the response to TMPEA. All intestinal tissue was allowed to equilibrate in the bath at least 30 min. before commencement of any challenge. In some experiments, guinea pig ileum from fasted animals was removed, quick frozen (dry ice and acetone), and placed in the cold (5°) for periods of 24 to 60 hr. before being challenged with TMPEA. Tissue which had been frozen was allowed to equilibrate for 2 hr. prior to experimentation. The object of these experiments was to observe the effect of TMPEA on denervated smooth muscle. The doses of all compounds are expressed as their salts. The drugs and their range of concentration (mcg./ml.), in terms of base, are given in Table I.

TABLE I—RANGE OF CONCENTRATION OF COMPOUNDS *In Vitro*

Compd.	Range of Dose Employed (Base mcg./ml.)
Mescaline sulfate	0.017–140.0
Phenoxybenzamine·HCl	2.2–8.8
Isopropylmethoxamine·HCl	0.86–43.0
Hexamethonium chloride	0.92–3.70
Tetraethylammonium chloride	4.35–43.5
Serotonin creatinine sulfate	0.0004–2.1
Nicotine (pure)	1.0
Ergotamine tartrate	0.44–2.20
l-Epinephrine-*d*-bitartrate	0.014–2.75
l-Phenylephrine·HCl	0.010–4.10
Acetylcholine chloride	0.009–0.045
Atropine sulfate	0.0083–4.15
Hydralazine·HCl	4.05–8.10
Reserpine	1.0–12.5
Isoproterenol·HCl	0.0043–0.043
Dichloroisoproterenol	0.0087–8.7
Pilocarpine·HCl	8.5–10.6
Histamine diphosphate	0.18
Lidocaine·HCl	43.5–261.0
l-Arterenol-bitartrate	0.013–2.65
Procaine·HCl	43.5–261.0
Diphenhydramine·HCl	0.08–0.44

RESULTS

Effect of TMPEA on the Intestinal Tissue of Several Species—During the course of this research, it became apparent that the action of TMPEA on the terminal section of ileum was concentration, species, and tissue dependent. Over a wide range of concentration, the effect of TMPEA on guinea pig and cat ileum was consistently motor in nature, while concentrations approaching the upper limit were necessary for responses of equal magnitude for the rat ileum. In general, in all species concerned, the lowermost portions of the ileum were more responsive to the motor stimulatory property of TMPEA than were portions adjacent to the jejunum. In fact, the terminal portion of the ileum was the segment of intestinal tissue most sensitive to the effects of TMPEA. It was noted that the motor response to TMPEA roughly paralleled the motor response to norepinephrine, epinephrine, and serotonin. The response to TMPEA of other intestinal tissue (*e.g.*, duodenum, jejunum, and upper portion of ileum) was considerably less than that observed for the terminal segment of the ileum in all species employed. The effect of TMPEA on isolated tissue from various sources, including the present study, is presented in Table II.

Effect of Diphenhydramine Pretreatment on the Response to Mescaline—Diphenhydramine, in concentration sufficient to block the motor response to histamine on guinea pig ileum, was ineffective against TMPEA. Similar results were obtained during an *in vivo* phase of this work (38).

Effect of Mescaline on Cholinergic Mechanisms—In order to demonstrate whether TMPEA could be

[1] NaCl, 120 Gm.; $CaCl_2$, 3 Gm.; KCl, 3 Gm.; $NaHCO_3$, 15 Gm.; glucose, 15 Gm.; $MgCl_2 \cdot 6H_2O$, 1.5 Gm.; and $NaHPO_3$, 0.75 Gm./15 L.

[2] NaCl, 60 Gm.; $CaCl_2$, 0.9 Gm.; KCl, 6.3 Gm.; $NaHCO_3$, 7.5 Gm.; glucose, 3.75 Gm.; $MgCl_2 \cdot 6H_2O$, 0.1597 Gm.; sucrose, 879 Gm./15 L.

[3] Brooks Sho rate flow meter, Brooks Instrument Co., Inc., Hatfield, Pa.

TABLE II—EFFECT OF MESCALINE (I), PRETREATMENT WITH DRUGS BEFORE MESCALINE (II), AND EFFECTS OF DRUGS ON MESCALINE ACTIVITY (III) ON SELECTED TISSUES OF VARIOUS SPECIES *In Vitro*[a]

Species	Tissue	I	mcg./ml.	II[c]	III[c]
Guinea pig	Duodenum	Sl. contraction	10–50	...	...
	Jejunum	Sl. contraction	10–50	...	...
	Ileum (terminal)	Contraction; magnitude depends upon initial tissue response	0.5–50	Acetylcholine (SA); L-epinephrine (A, R); L-norepinephrine (A, R); isoproterenol (NA); 5-HT (V; SA, A); nicotine (NA); phenylephrine (A)	Procaine (NA); lidocaine (NA); hexamethonium (SE); TEA (SA); atropine (NA); reserpine (NA, A); diphenhydramine (SA); DCI (NA); phenoxybenzamine (I); hydralazine (V); methoxamine (V); ergotamine (NA); nicotine (NA)
	Uterus	Variable (all or none type)	12.5–5	5-HT (I)	Reserpine (NA, I); DCI (V); hydralazine (V); methoxamine (V); ergotamine (NA)
		No effect (1)	10–200		
	Tracheal chain	Contraction	50–200	L-Epinephrine (SR)	...
	Aorta	Sl. contraction	50–100	L-Epinephrine (NA); L-norepinephrine (NA)	...
Rat	Ileum	Contraction	50	L-Epinephrine (SR); L-norepinephrine (SR); phenylephrine (SR)	DCI (V)
	Uterus	Contraction, increase in pendular movement	0.1–50	5-HT (NA) (3); 5-HT (S) (2) 5-HT (A); L-epinephrine (A, R); L-norepinephrine (A, R); ergotamine (NA); methoxamine (NA)	Atropine (NA) (2); hydralazine (V); methoxamine (NA); ergotamine (NA); DCI + TMPEA nullified the effect of epinephrine
		No effect	10–200		
		Contraction (3)	50		
		Contraction (2)	0.1–0.4		
Cat	Duodenum	No effect (1)	10–400	...	...
	Ileum	Contraction	12.5–50	...	Phenoxybenzamine (I)
	Uterus	No effect (1)	10–200	...	...
	Nictitating membrane	No effect	50–200	...	...
	Atria	No change in chronotropy or inotropy	12.5–50	Acetylcholine (SA); L-epinephrine (SE); L-norepinephrine (SE); isoproterenol (SE)	...
Rabbit	Duodenum	No effect (1)	10–400	...	...
	Uterus	No effect (1)	10–200	...	...
Human	Placental vessels	Constriction[b] (4)		5-HT (S) low dose 5-HT (no effect) high dose Epinephrine (no effect) high dose	...

[a] Concentration of compounds expressed as their salts. [b] Perfusion. [c] Key: A = antagonism, I = inhibition, V = variable, SA = slight antagonism, NA = no antagonism, SE = synergism (excitatory effect), SR = synergism (relaxatory effect), R = response reversal.

blocked by atropine, the tissue (guinea pig ileum) was first challenged with a submaximal concentration of acetylcholine (ACH), and when the response was maximal, atropine was added. After 2 or 3 min., another challenge to ACH was performed and if no response was elicited TMPEA was added to the system. On 30 occasions the characteristic motor response to TMPEA was observed. It has been reported (10) that TMPEA possesses a curare-like action. A series of experiments was performed to observe the action of TMPEA on ACH induced contractions. Tissue which did not actively respond to mescaline (25–50 mcg./ml.) did not block ACH contractions on the guinea pig ileum. If a tissue contracted in the presence of TMPEA, ACH produced a response which was less than control. However, if the heights of the induced TMPEA and ACH contractions were added; it equaled control response. The same phenomenon was observed with the catecholamines. Therefore, the specificity of the reaction is questionable.

Effect of Local Anesthetics, Lidocaine and Procaine, on the Activity of Mescaline—Concentrations of the above local anesthetics (50 to 250 mcg./ml.) not sufficient to entirely inhibit the action of ACH did not affect the response to TMPEA on the guinea pig ileum. Higher concentrations (300 mcg./ml.) were not observed to curtail the action of TMPEA, although these concentrations did block the action of ACH.

Effect of the Depolarizing Ganglionic Blocking Agent, Nicotine, on the Response to Mescaline in the Guinea Pig Ileum—Nicotine (1 mcg./ml.) did not antagonize the action of TMPEA (25 mcg./ml.) on the guinea pig ileum. Reversal of the procedure yielded the same results. In the majority of trials, nicotine was allowed a 3-min. contact period prior to the challenge with TMPEA.

Effect of TMPEA on the Adrenergic Receptor—Studies were initiated to observe the possible interaction between TMPEA and adrenergic receptors. Ergotamine, in sufficient concentrations (2.5–5.0 mcg./ml.) to block the motor response to a catecholamine (norepinephrine or epinephrine) was not observed to inhibit the motor response to TMPEA on guinea pig ileum and rat uterine tissue. It was noted that epinephrine and norepinephrine caused a greater degree of relaxation in the presence of *both* ergotamine and TMPEA, than in the presence of ergotamine only. Subsequently, it was observed that TMPEA *alone* could not only block the motor action of a catecholamine but also allow the relaxatory action to be manifested. In general, if the catecholamines produced weak to moderate motor responses, TMPEA was capable of antagonizing these responses. However, if a catecholamine caused a marked motor effect and TMPEA did not generate its characteristic action, the antagonism was diminished. It was apparent that sufficient incubation time and the concentration of TMPEA

was essential to its ability to effectively antagonize the catecholamines. An incubation period of 5 min. was found to be necessary for reproducible responses; however, other factors, such as the responsiveness of the tissue to both the catecholamines and TMPEA, were of equal importance. If shorter incubation periods were employed, the antagonism was proportionally diminished. Furthermore, it was demonstrated that when TMPEA was added after a "critical" period of time had elapsed following a stimulatory dose of norepinephrine, epinephrine, or phenylephrine (5 min.) an additional increase in tone was produced. If TMPEA was added before 5 min. had elapsed (1–3 min.) a decrease in tension was produced.

Interaction Between TMPEA and Serotonin, on Guinea Pig Ileum and Rat and Guinea Pig Uteri—TMPEA in very low concentration has been reported to synergize the motor action of serotonin on the rat uterus (2). Higher concentrations have been reported to have little effect on the activity of serotonin on rat uterus. The apparent synergistic action of low concentrations of TMPEA (0.1–0.4 mcg./ml.) observed during the present study on estrogen dominated uterus appeared questionable. Slightly higher concentrations of TMPEA (2.5 mcg./ml.) did antagonize the effect of serotonin. TMPEA was also observed to antagonize the effect of serotonin (100 trials) on guinea pig ileum. The degree of antagonism depended upon the sensitivity of the tissue to both compounds. Moderately stimulatory doses of serotonin could be almost completely blocked with very high concentrations of TMPEA. Ergotamine and TMPEA acted synergistically to antagonize the action of serotonin. In addition, moderate doses of TEA (15 mcg./ml.) plus TMPEA acted synergistically to antagonize the motor response to serotonin. TMPEA (25 mcg./ml.), after a 3-min. incubation period, was observed to block the motor response of guinea pig uterus to serotonin (0.25 mcg./ml.). Additional challenges were effectively antagonized.

Effect of TMPEA on Guinea Pig Tracheal Tissue—TMPEA alone (50–200 mcg./ml.) or following a pilocarpine (10 mcg./ml.)-induced spasm caused contraction. Epinephrine provoked relaxation alone or after either of the above procedures. The relaxation due to epinephrine appeared to be greater following mescaline than when the tissue was not pretreated. Unfortunately, a great deal of variability was encountered with this tissue, obviating definite conclusions.

The Interaction of TMPEA with Various Adrenergic Blocking Agents—A number of experiments were conducted to observe the possible interaction between hydralazine, isopropylmethoxamine, dichloroisoproterenol, and mescaline in various tissue. Concentrations of dichloroisoproterenol (10 mcg./ml.) were observed to inhibit or reduce the motor activity produced by TMPEA, while lower concentrations (0.5 mcg./ml.) had little effect. All tissue tested (guinea pig ileum and uterus and rat ileum and uterus) with the exception of cat atria reacted similarly. Hydralazine and isopropylmethoxamine had little modifying action on tissue response to TMPEA. However, it was noted that concentrations of TMPEA which were usually ineffective would produce a pronounced action on guinea pig uterus if the tissue were pretreated with either hydralazine or isopropylmethoxamine. In general, the results of the above procedures were difficult to assess because of the strong motor activities inherent to hydralazine and isopropylmethoxamine.

Concentration of DCI (10 mcg./ml.) and TMPEA (25–50 mcg./ml.) were effective in blocking the action of the catecholamine on rat uterine tissue. These results indicated that both α- and β-receptor sites were blocked. A more potent α-adrenergic blocking agent, phenoxybenzamine (10–20 mcg./ml.), was observed to completely block the motor activity due to TMPEA (25–50 mcg./ml.) on cat and guinea pig ileum. These results would suggest that TMPEA exerts its effect on the α-adrenergic receptor site.

In order to substantiate these results and to demonstrate the action of TMPEA on the β-adrenergic receptor site, cat atria (right atria) were selected as the test structure. TMPEA (12.5–50 mcg./ml.) had no demonstrable chronotropic or inotropic effect on this tissue, even after a 10-min. incubation period. Nevertheless, the tissue remained responsive to various catecholamines, isoproterenol, epinephrine, and norepinephrine. Pretreatment of the tissue with TMPEA (25–50 mcg./ml.) caused an apparent synergistic effect to the catecholamines. Phenoxybenzamine (10 mcg./ml.), incubated for the same period of time as TMPEA (3 min.), also seemed to synergize the action of the catecholamines. In contrast, DCI (10 mcg./ml.) completely blocked the action of isoproterenol, and to a lesser extent, epinephrine and norepinepheine. The synergistic effect of TMPEA was best observed if the water bath temperature was reduced from 37.5° to 32.0°.

It was of interest to observe the effect of TMPEA on ACH blockade of the right atria, in view of a report that TMPEA possesses a curare-like action. The effect of complete or partial blockade of normal rhythmicity of atrial tissue by ACH (0.25–0.5 mcg./ml.) was not affected by pretreatment with TMPEA (25–50 mcg./ml.). When slight antagonism was observed, the tissue usually responded in a different fashion to ACH following the washout of TMPEA. The very slight antagonism encountered in this series might be explained more logically as due to a change in response of the tissue to ACH, rather than specific antagonism by TMPEA.

DISCUSSION

Diphenhydramine, in sufficient concentration to block histamine, did not effectively antagonize TMPEA. There is some doubt as to how antihistamines actually antagonize histamine (16). This fact precludes a definitive conclusion that mescaline does not exert its effect *via* histaminic receptors. However, graded responses caused by TMPEA in the presence of diphenhydramine were not observed. These findings would suggest that a noncompetitive reaction due to the effect of TMPEA prevailed and the response elicited was distinct from the histamine effect.

Costa (2) reported that atropine *in vitro*, in sufficient concentration to block ACH, did not antagonize the effect of TMPEA in rat uterus. Grace (1) reported that atropine, *in vivo*, was capable of blocking the effect of TMPEA in the cat. The results obtained in this study using guinea pig

ileum are in agreement with those of Costa (2). Atropine in sufficient concentration to block submaximal stimulatory effects of ACH did not inhibit the motor response induced by TMPEA. Thus, TMPEA apparently does not produce its motor effects *via* a muscarinic action.

In order to determine if TMPEA stimulated nervous elements, local anesthetics were employed in concentrations which block ACH. The dose for both procaine and lidocaine, necessary to block ACH, caused a marked increase in the activity of the ileum. TMPEA was able to exert its effect in the presence of those compounds. These findings, in conjunction with those above, support the concept that TMPEA does not exert its effect on cholinergic receptors.

Ganglionic blocking agents (hexamethonium and TEA) did not antagonize the effect of TMPEA on guinea pig ileum. In the presence of both a ganglionic blocking agent and atropine, mescaline was observed to produce its characteristic effect. Thus it appears that TMPEA does not exert its action on ganglia. Moreover, large concentrations of TEA seemed to synergize the motor response exerted by TMPEA and serotonin. The latter findings are in agreement with those reported by Day and Vane (17) for hexamethonium.

Gaddum *et al.* (3), in an attempt to explain the antagonizing action of LSD on serotonin, postulated two distinct types of receptors in ganglia cells in the intestine. One of these was stimulated by ACH or nicotine and inhibited by excess nicotine or hexamethonium, while the other type was stimulated by serotonin and inhibited by excess serotonin. It is also possible that there exists two types of cells; one stimulated by nicotine, the other by serotonin. In light of this theory, TMPEA might conceivably be stimulating serotonin-sensitive receptors. However, serotonin was not observed to alter the action of TMPEA on guinea pig ileum. It is possible that the concentrations employed in these experiments were not sufficient to antagonize serotonin receptor sites.

Gaddum *et al.* (3) further differentiated between the response of smooth muscle of the ileum and rat uterus to serotonin. They stated that the receptors in the rat uterus are easily paralyzed by excessive serotonin, whereas those in the ganglia of the guinea pig ileum are not. They explained the antagonistic action of LSD on rat uterine tissue on the above hypothesis. TMPEA was not observed, in their studies, to antagonize the effect of serotonin on rat uterus. Costa (2) reported that mescaline acted synergistically with serotonin in causing increased activity on rat uterine tissue. Savini (18) reported that LSD was antagonistic to serotonin-induced constriction on perfused rabbit ear. Prior to Gaddum's theory concerning the site of action of serotonin, Rocha e Silva *et al.* (19) postulated the site to be the postganglionic cholinergic nerve fiber of the ganglia. Cocaine has been observed to effectively antagonize serotonin on isolated ileum, a finding which supports the proposal of Rocha e Silva and his collaborators.

Day *et al.* (17) have presented evidence demonstrating that serotonin is also capable of stimulating smooth muscle directly. However, they consider the blockade of the action of serotonin by phenoxybenzamine to be nonspecific and concluded that serotonin primarily exerts its effect indirectly through stimulation of cholinergic nerve fibers in guinea pig ileum.

The findings in this study involving the interaction between serotonin and TMPEA on guinea pig ileum and uterus and rat uterus are in agreement with the theory proposed by Gaddum *et al.* (3) and the findings of Day *et al.* (17). In the majority of experiments, there appeared to be some antagonism exerted by mescaline on the motor response to serotonin on the guinea pig ileum. These observations would indicate that the ganglionic component involved in the action of serotonin was not effectively antagonized, while the smooth muscle was affected.

Experiments conducted on rat and guinea pig uteri clearly demonstrated that TMPEA effectively antagonized the action of serotonin. These results are not in agreement with those reported by Gaddum *et al.* (3) and Astrom *et al.* (4). The latter authors stated that TMPEA had "no certain action against serotonin on human placental vessels." The findings in the present investigation would support the variation in receptor sites in different tissues sensitive to serotonin. They would also support the concept that TMPEA was primarily exerting its effect on the smooth muscle cell. These results support the clinical and experimental findings involving animals demonstrating cross-tolerance between LSD and TMPEA.

Costa (2) and Astrom (4) reported that low concentrations of TMPEA synergized the effect of serotonin on rat uterus and human placental preparations, respectively. Costa's (2) experiments were repeated precisely as he reported them during this investigation. After reproducible responses were obtained from challenges with serotonin by the method of Rocha e Silva (19), the tissue was challenged with TMPEA. When serotonin was added to this system, a synergistic effect was apparent. However, if this experiment was repeated without the addition of TMPEA, the same effect was observed. Thus the augmented response due to serotonin in the presence of TMPEA, in this investigation, appears to have been a function of variability in response to serotonin and not to a synergistic action of TMPEA.

Nicotine was not observed to antagonize the effect of TMPEA. It is generally agreed that nicotine depolarizes the postsynaptic neuron in ganglia, making it insensitive to acetylcholine (20, 21). Emmelin and Feldberg (22) demonstrated that nicotine also depressed smooth muscle directly. Evans and Schild (23) demonstrated that nerve-free preparations of intestine reacted to nicotine by contraction. This effect could be abolished by pretreatment with hexamethonium. The latter substance was reported to be the most specific ganglionic blocking agent known (24). The ganglionic blocking agents, *i.e.*, the depolarizing and nondepolarizing competitive types have, therefore, been shown to possess a number of properties which could have influenced the activity of TMPEA. However, if their major activities are considered, one would necessarily conclude that TMPEA does not possess characteristics of a substance which excites intramural ganglia cells responsive to ACH. Grace (1) reported that TMPEA caused an increase in tonic activity of uteri, *in situ*, from various species. The

effect was not abolished after pretreatment with nicotine.

TMPEA has been noted, in this study and others (1–3), to induce rhythmic movements in intestinal and uterine tissue. Various investigators have attributed the rate of spontaneous activity of uterine tissue to the level of catecholamine content (25, 26). Rudzik *et al.* (25) stated that the initial rate of spontaneous uterine contractions *in vitro* was inversely proportional to the quantity of epinephrine in the uterus. When the uterus from a rat in estrus was challenged with epinephrine, relaxation occurred—TMPEA caused contraction. The possibility exists that TMPEA produces uterine contraction by causing a release of endogenous epinephrine or directly stimulating excitatory sites, while epinephrine may relax the tissue by raising the endogenous level of epinephrine content or directly stimulating inhibitory sites.

The terminal segment of the ileum in a variety of species has been observed to contract in response to splanchnic nerve stimulation or to exogenous epinephrine (27–30). Dale (27) utilized this effect to demonstrate that the ergot alkaloids blocked the contracting properties of epinephrine on the terminal segment of the cat ileum. Munro (28) reevaluated this phenomenon and showed that various catecholamines were capable of causing similar responses (29). This motor effect produced by epinephrine on the intestinal tissue was not in accord with the theory proposed by Ahlquist (31). At that time α activity on intestinal tissue was classified as relaxatory. Some changes in this classification were proposed by Lands (32). He demonstrated that inhibitory and excitatory receptors were present in the terminal segment of the ileum and the response obtained was probably a function of the relative number and sensitivity of each type of receptor present. Furchgott (33) expanded upon the above classification and introduced the term "delta receptor" to explain intestinal inhibition mediated by a sympathomimetic amine. The subject, at present, is still unsettled (34).

In the present investigation, it was observed that when the terminal section of ileum was relaxed by a catecholamine, TMPEA had little or no effect. If a contraction was elicited in the presence of a catecholamine, TMPEA alone was also observed to produce a similar response. Moreover, the more sensitive the tissue appeared to be toward the motor effect of the catecholamines (contraction), the more sensitive it was to the effects of TMPEA. The above actions were interpreted as demonstrating that both receptors (α and β) or A_c (excitatory) and A_r (relaxatory) were present in the terminal segment of the guinea pig ileum. If a contraction was elicited, the α responses predominated, or if inhibition resulted, the β responses were manifested.

Ergotamine in sufficient concentration to antagonize the motor responses to epinephrine and norepinephrine did not inhibit the motor response to TMPEA on guinea pig ileum and rat uterine tissue. Phenoxybenzamine was capable of blocking the motor response of TMPEA on guinea pig and cat ileum as well as reversing a former stimulatory action of the catecholamines to a purely relaxatory one. The apparent ability of phenoxybenzamine and not ergotamine to effectively antagonize TMPEA may be attributed to the lower concentrations employed with the latter compound. Ergotamine and TMPEA appeared to possess synergistic properties in antagonizing motor responses to the catecholamines. The results would indicate that TMPEA was stimulating excitatory receptors in the terminal section of guinea pig ileum. Abolishment of this effect by phenoxybenzamine and the apparent synergistic effects of ergotamine and TMPEA on the blockade of motor responses to the catecholamines strongly suggest that TMPEA exerts its effect on α-adrenergic receptor sites.

Supporting this concept are the findings that TMPEA alone is capable of antagonizing the motor response to catecholamines in guinea pig ileum, rat ileum, and uterine tissue. Furthermore, simultaneous administration of dichloroisoproterenol (a β-adrenergic blocking agent) and TMPEA abolished the effect of epinephrine on rat uterus, thus demonstrating that both α- and β-adrenergic receptor sites were blocked. If TMPEA was stimulating α-adrenergic receptor sites, it should theoretically be possible to demonstrate competition between these compounds. This competitive phenomenon was observed to occur when TMPEA was added to a preparation which had reacted (motor response) to a catecholamine. It was also observed that TMPEA had to be introduced within a critical period of time, otherwise competition was not observed. These findings are in agreement with those reported by Speck (7) on the competitive nature existing between epinephrine and TMPEA on various parameters in the rat.

Experiments were conducted to observe a possible augmentation of the effect of mescaline with the use of β-adrenergic blocking agents. Of the three agents employed, isopropylmethoxamine, hydralazine, and dichloroisoproterenol, the latter was the most specific. The former compounds are also known to possess α-adrenergic stimulatory actions (34). The results would indicate that the β-adrenergic blocking agent, dichloroisoproterenol, did augment the action of TMPEA on guinea pig uterus. If isopropylmethoxamine was administered after TMPEA produced an excitatory response in rat uterus and ileum or guinea pig ileum, a marked reduction in tone was noted. These results would indicate that the former compound was exerting its effect on β-adrenergic sites, if the assumption is made that TMPEA had occupied the α-adrenergic sites. The variability encountered in these experiments, however, does not justify definitive conclusions. Powell and Slager (14) demonstrated that dichloroisoproterenol blocked the relaxatory response of epinephrine on tracheal tissue. Substituting TMPEA in place of dichloroisoproterenol did not alter the relaxatory response of epinephrine. These findings further substantiate the concept that TMPEA is not exerting an appreciable effect on β-adrenergic receptor sites. The fact that TMPEA increased the tone of the tracheal chain affords additional evidence that its activity is primarily on α-adrenergic receptor sites.

Reserpine, in low concentrations, was not observed to antagonize the action of mescaline on guinea pig ileum. Higher concentrations did effectively antagonize the motor activity of mescaline on guinea pig ileum and uterine tissue. The latter effect might be due to a direct action on the smooth muscle. The higher concentrations employed in

these studies have been observed to markedly reduce the response of guinea pig ileum to ACH and histamine (35). Reserpine is known to release catecholamines from isolated tissue (36). These effects might nonspecifically antagonize the action of TMPEA.

Chronotropic and inotropic changes were not observed to occur in isolated right atria (cat) in the presence of TMPEA. Similar results were obtained with phenoxybenzamine. The latter findings are in agreement with those of Nickerson and Chan (37). Changes in rate and force of contraction have been attributed to β-adrenergic stimulation (33, 34). Dichloroisoproterenol was observed, in this study and by Nickerson *et al.* (37), to antagonize the effect of various catecholamines on cardiac tissue. TMPEA did not, however, reduce catecholamine activity. There also appeared to have been some augmentation to the simulatory effects of epinephrine, norepinephrine, and isoproterenol in the presence of TMPEA. These results are supportive of the findings with TMPEA on other tissue used in this study; that is, TMPEA exerts its primary action on α-adrenergic receptor sites.

TMPEA has been reported to possess a curare-like action (10). In this study, TMPEA was observed to apparently antagonize the effect of ACH on smooth muscle of the guinea pig ileum and cat atria. The latter experiments were too variable to draw any conclusions. However, epinephrine and norepinephrine were also observed to diminish the response of smooth muscle to ACH. These results would indicate that the antagonizing effect of TMPEA on ACH-induced contractions was nonspecific. The fact that Schopp *et al.* (10) reported that epinephrine and prostigmine oppose the paralyzing action of TMPEA supports this concept.

CONCLUSIONS

Evidence is presented which demonstrates the ability of TMPEA to stimulate α-adrenergic receptor sites in various peripheral tissue. The fact that TMPEA alone can antagonize α-stimulatory agents, and in combination with specific β-blocking agents can annul the effects of epinephrine, strongly suggest that it possesses α-adrenergic blocking properties. Furthermore, since it is capable of contracting smooth muscle preparations containing excitatory receptors, and because its actions were inhibited by α-adrenergic blocking agents, the concept that TMPEA possesses α-stimulatory properties is demonstrated. Competition between TMPEA and various catecholamines provides additional evidence that TMPEA possesses agonistic and antagonistic properties. The β activity of TMPEA appeared to be slight. To clarify this action, TMPEA should be studied on target structures which possess a preponderance of β-adrenergic receptors.

Evidence is presented that TMPEA does not stimulate cholinergic receptors. Clarification of the action of TMPEA on receptors in the ganglia should be carried out with more specific ganglionic blocking agents and with techniques reported to reduce ganglionic sensitivity. TMPEA has been shown to antagonize effectively the action of serotonin on guinea pig and rat uteri, but not in guinea pig ileum. It is concluded that in the latter tissue serotonin exerts an effect on ganglionic receptors which cannot be blocked by TMPEA.

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Keyphrases

Mescaline (trimethoxyphenylethylamine)
In vitro action on smooth muscle
Cholinergic mechanism—effect on
Adrenergic receptor—effect on
Adrenergic blocking agents—interaction mescaline
Serotonin—mescaline interaction
Local anesthetic effect on mescaline activity
Nicotine effect on mescaline activity
Diphenhydramine effect on mescaline activity