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BLOCKADE OF THE GANGLIONIC STIMULANT ACTION OF 5-HYDROXYTRYPTAMINE

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Blocking actions of drugs antagonistic to 5-hydroxytryptamine (5-HT) have been investigated almost exclusively on smooth muscle organs either *in vitro* or *in vivo* (Gyermek, 1961). The possible role of 5-HT in the functions of the nervous system makes it desirable to study the interactions between 5-HT and its antagonists on more appropriate biological systems. The observations of Gaddum and Picarelli (1957) on the isolated guinea-pig ileum called attention to the different pharmacological behavior of certain 5-HT receptors. Some of these receptors were postulated to be nervous (M) receptor types, sensitive to blocking agents such as morphine, cocaine and atropine; others, muscular (D) receptor types sensitive to lysergic acid diethylamide, phenoxybenzamine (Dibenzyline) and benzyloxygramine. However, by using the isolated ileum preparation, it was not possible to elucidate the nature of the nervous receptors sensitive to 5-HT. On the guinea-pig ileum, 5-HT may act in different ways; *i.e.*, on viscero-sensory receptors (Mott and Paintal, 1953), on intramural ganglia, and also, through these elements at the level of the parasympathetic effector sites as well as on the smooth muscle directly. Recent studies on the cat superior cervical ganglion-nictitating membrane preparation (Trendelenburg, 1956, 1959), on the dog pelvic nerve-bladder preparation (Gyermek, 1960) and on chemoreceptors (Ginzel and Kottegoda, 1953), suggested that 5-HT produces a direct stimulation of ganglionic receptors. The aim of this study was to analyze the antagonistic actions of some well-known blocking agents of 5-HT on a purely nervous, ganglionic receptor sensitive to 5-HT.

METHODS. The inferior mesenteric ganglion was prepared as described by Herr and Gyermek (1960). Cats were anesthetized with a mixture of 60 mg/kg chloralose and 250 mg/kg urethane i.p. The intestines were removed; the abdominal cavity was washed with saline and filled with warm, light mineral oil. The inferior mesenteric artery was cannulated for close intraarterial injection to the ganglion. The right, postganglionic (hypogastric) nerve was dissected, cut distally, and desheathed. Generally, small bundles of fibers were prepared but in some cases single fibers were isolated. The cut postganglionic nerves were placed on a bipolar platinum electrode. Spontaneous activity was recorded by means of a Tektronix Type 532 oscilloscope with a 122 DC preamplifier. Continuous moving film records were obtained from a slave scope with a Grass Kymograph camera.

1,1 - Dimethyl,4 - phenylpiperazinium iodide (DMPP (Parke, Davis & Co.)) and 5-HT as creatinine sulfate complex (Sandoz) were used as ganglionic stimulants. At the onset of each experiment, these agents were given in varying doses to determine the equipotent doses that would be used as challenging doses against the antagonists for the remainder of the experiment. All injections of both stimulants and blocking agents were given in 0.4 ml saline during a 10-second interval. The following blocking agents were used: morphine sulfate, cocaine HCl, atropine sulfate, Dibenzyline HCl (Smith Kline & French), lysergic acid diethylamide, 2-bromolysergic acid diethylamide (Sandoz), benzyloxygramine (Regis) and hexamethonium dichloride.

Blocking agents were administered at two- to four-dose levels. Doses of the stimulant drugs, 5-HT and DMPP, were always repeated twice after administration of the blocking agent—once after 30 seconds (which proved to be the optimal time interval for obtaining blockade), and again after 4 minutes. Because of the delayed onset of the adrenolytic action of Dibenzyline, records were also taken with this compound 10 and 15 minutes after administration. After complete recovery from the blockade, the same dose of blocking agent was again administered and the second of the two stimulants given. The sequence of the stimulants was alternated from one experiment to another. Doses of the stimulant and blocking agents are given in terms of salts.

RESULTS. I. STIMULANT ACTION OF 5-HT AND DMPP ON THE INFERIOR MESENTERIC GANGLION. On intraarterial injection, 5-HT and DMPP produced marked increase of the postganglionic nerve activity 3 to 7 seconds after completion of the injection.

A. *The role of preganglionic activity.* Recordings from preganglionic fibers during injection of the two stimulants showed no change in preganglionic efferent activity.

In a ganglion isolated *in situ*, after cutting all pre- and postganglionic nerves, spontaneous activity markedly decreased or even ceased, but a marked increase in activity was evoked after injection of the two ganglionic stimulants.

B. *Stimulant potency.* On multifiber preparations the effective dose range producing ganglionic stimulation was found to be 1.25 to 10 μg for 5-HT, and 0.5 to 2 μg for DMPP. In general, 5-HT was one-fifth as potent as DMPP. Comparison of the ganglionic stimulant actions of 5-HT and DMPP on a single fiber preparation is demonstrated in the first figure. On the basis of the dose-frequency response correlations, 5-HT is 2.5 times less potent than DMPP.

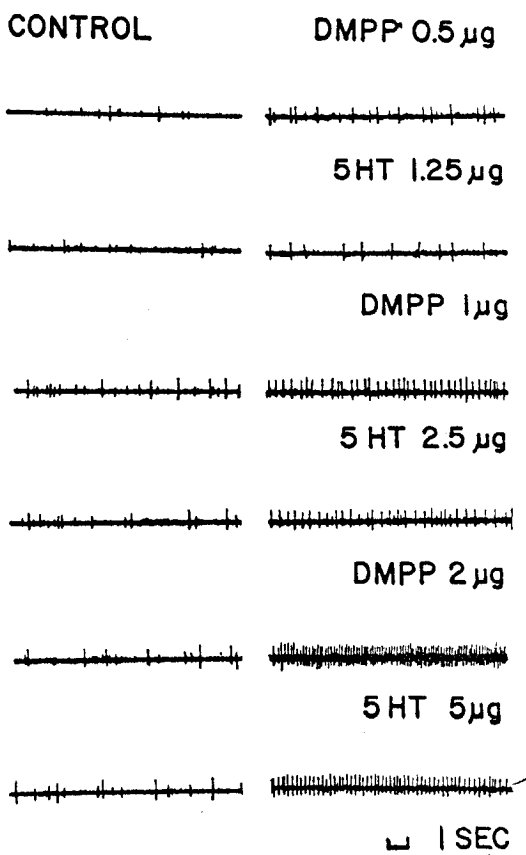

FIG. 1. Stimulant effect of 5-HT and DMPP on the inferior mesenteric ganglion of the cat.
Single fiber preparation. Recordings on the left side of the figure are control periods; recordings on the right side are taken 3 to 5 seconds after the drugs have been administered. Note the increasing number of spikes dependent upon the doses administered. (Record retouched.)

II. BLOCKADE OF THE GANGLIONIC STIMULANT ACTION OF 5-HT AND DMPP. Seven blocking agents which are known to antagonize the action of 5-HT on the muscular and nervous receptors of the guinea-pig ileum were used, and hexamethonium was employed as a cholinergic blocking agent. Characteristic sections of three experiments using different blocking agents are shown in figures 2, 3 and 4.

Figure 2 indicates that there is some blockade by morphine to 5-HT at the 40-μg level. The blockade was complete at 200 μg whereas no blockade to DMPP was seen until the 1-mg level was reached.

In contrast to morphine, LSD blocked 5-HT and DMPP to an equal extent. The minimum blocking dose in the experiment represented in figure 3 was 400 μg. Hexamethonium, in the dose range used (25 to 100 μg) was effective only against DMPP (fig. 4).

Results obtained with eight blocking agents are summarized in table 1. A diminution in action potential amplitude of 50% or more, occurring 30 seconds after the blocking drug was administered, was considered an effective blockade. The number of effective blockades obtained at different dose levels is represented.

A. *Potency against 5-HT.* Some agents, like morphine, cocaine, bromoLSD, and benzyloxygramine, usually produced either partial or complete blockade in the 40- to 200-μg dose range. Atropine and LSD were less potent, eliciting inhibition of the 5-HT effect in the 200-μg and 400-μg dose range, respectively. Dibenzyline was the least potent of the drugs which were considered to have anti-5-HT properties. It was only slightly effective at the 1-mg dose level. This weak action of Dibenzyline was observed after 30 seconds and did not increase after a longer period of observation. This is in contrast to the slow onset of its adrenolytic action. Thus, the order of potency of 5-HT antagonists investigated is as follows: morphine

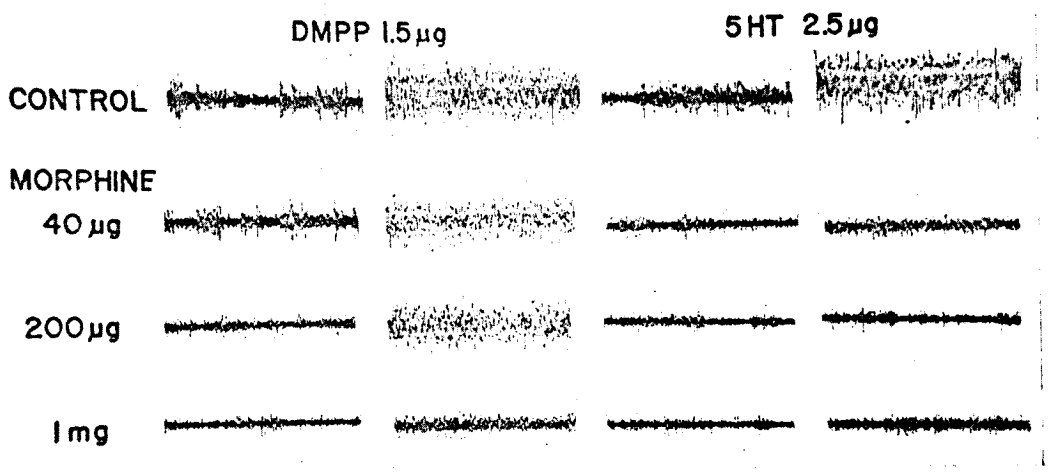

FIG. 2. The action of morphine on the ganglionic stimulation produced by DMPP and 5-HT. Multifiber preparation. Left two columns: ganglionic activity before and after 1.5 µg DMPP. Right two columns: ganglionic activity before and after 2.5 µg 5-HT. First line: without morphine. Last three lines: same reactions under the influence of 40, 200 µg and 1 mg morphine.

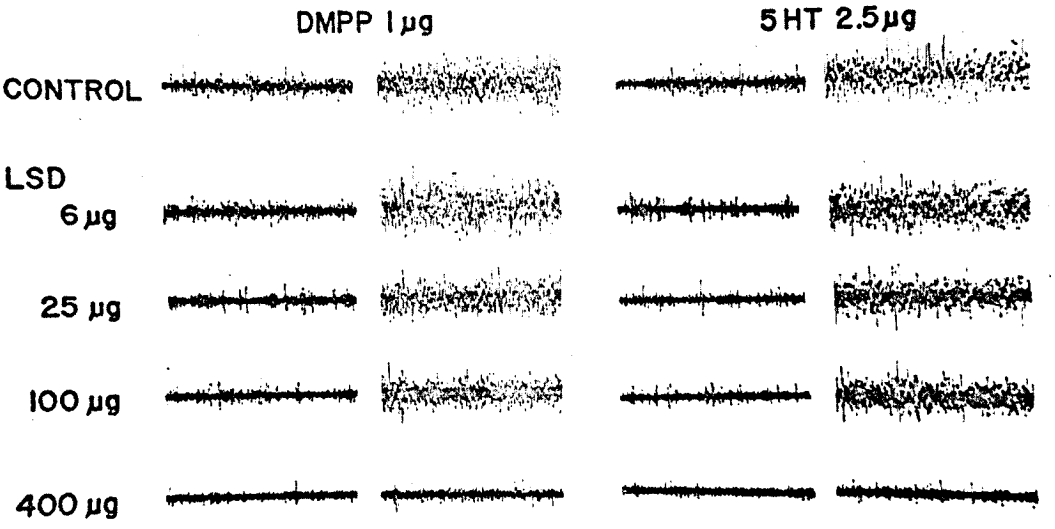

FIG. 3. The action of LSD on the ganglionic stimulation produced by DMPP and 5-HT. Multifiber preparation. Left two columns: ganglionic activity before and after 1.0 µg DMPP. Right two columns: ganglionic activity before and after 2.5 µg 5-HT. First line: without LSD. Last three lines: same reactions under the influence of 6, 25, 100 and 400 µg LSD. Stimulants are given 30 sec after LSD.

~ cocaine ~ bromoLSD ~ benzyloxygramine ~ LSD ~ atropine ~ Dibenzyline.

The blockade produced by these compounds was short lasting, and complete recovery (with the exception of benzyloxygramine) was usually obtained within 4 minutes.

B. *Potency against DMPP.* The most potent member of the series was hexamethonium, which blocked DMPP completely in a dose of 25 µg, followed by atropine and bromoLSD which were effective at the 200-µg level. LSD, benzyloxygramine and cocaine were less potent.

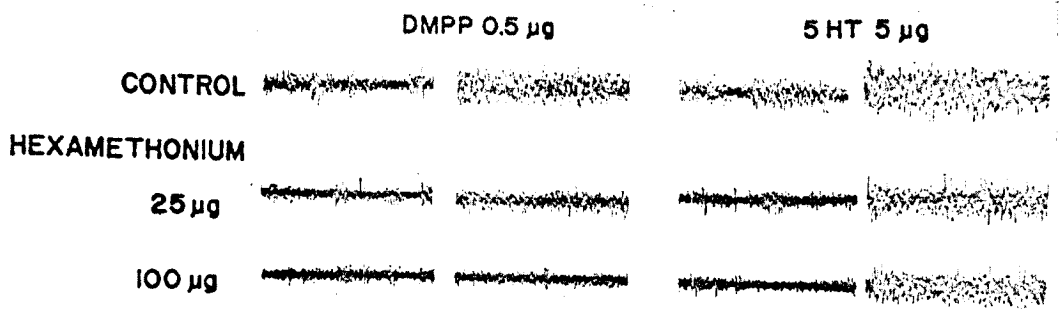

FIG. 4. The action of hexamethonium on the ganglionic stimulation produced by DMPP and 5-HT. Multifiber preparation. Left two columns: ganglionic activity before and after 0.5 μg DMPP. Right two columns: ganglionic activity before and after 5 μg 5-HT. First line: without hexamethonium. Last two lines: same reactions under the influence of 25 and 100 μg hexamethonium. Stimulants are given 30 sec after hexamethonium.

Morphine and Dibenzyline were relatively very weak against DMPP. Blockade with these agents occurred only at the 1-mg level.

C. *Selectivity of the blockade.* As shown in table 1, the blocking potencies against 5-HT and DMPP do not run parallel. Morphine exhibited quite marked selectivity to 5-HT. This effect is less pronounced with cocaine and bromoLSD. Practically no selectivity to any of the stimulants was obtained with LSD, Dibenzyline and benzyloxygramine. Atropine was slightly more potent against DMPP than 5-HT. Hexamethonium blocked only the effect of DMPP. The order of selectivity of these compounds to 5-HT is as follows: morphine > cocaine > bromoLSD > LSD ~ Dibenzyline ~ benzyloxygramine > atropine > hexamethonium.

DISCUSSION. The results of these experiments demonstrate a direct ganglionic stimulation by small doses of 5-HT, corroborating the findings of Trendelenburg (1959), who obtained indirect evidence on the cat superior cervical ganglion-nictitating membrane preparation. Furthermore, there is evidence that 5-HT facilitates ganglionic transmission in sympathetic ganglia of the cat (Trendelenburg, 1956) and rat (Hertzler, 1961). It also potentiates the effect of pelvic nerve stimulation on the urinary bladder of the dog (Gyermek, 1960) and the effect of the chorda tympani stimulation in the dog (Petrucelli *et al.*, 1961). On the other hand, it has been reported that 5-HT depresses transmission in the ciliary ganglion of the dog but this finding was based only on a single observation and using a relatively high dose of 5-HT (Page and McCubbin, 1953). Although most of the observations demonstrate ganglionic stimulant action, the possibility for a depressant action on ganglionic transmission in certain ganglia might be considered unless the opposite can be proved.

In the light of the observations which are available, it seems that 5-HT has an influence not only in modulating, but also in eliciting, nerve impulses in the efferent pathways of the autonomic nervous system. It is also demonstrated that the 5-HT *receptors* of the inferior mesenteric ganglion are different from the cholinergic ganglionic receptors. Trendelenburg (1959) has reported a similar distinction in the cat superior cervical ganglion. The present findings indicate that the *receptors* of the sympathetic ganglia sensitive to 5-HT are unlike the 5-HT receptors of smooth muscle organs. The ganglionic *receptors* of the inferior mesenteric ganglion are not very sensitive to LSD and the lack of selectivity observed with this agent suggests that it should not be considered a neurotropic HT antagonist (Bindler and Gyermek, 1961).

The classification of anti-5-HT agents in D and M receptors (Gaddum and Picarelli, 1957) is not fully supported in this work if one takes into consideration the fact that the synaptic *receptor* sites of the sympathetic ganglion are analogous with the M receptors of the gut. However, it is possible that the type of nervous *receptors* with presumed 5-HT sensitivity varies with the organ system studied. Another possibility for differences between this preparation and the guinea-pig ileum is that the latter contains cholinergic postganglionic nerve endings as a site of action of a blocking agent such as atropine.

TABLE 1

Comparison of different blocking agents to 5-HT and DMPP on the inferior mesenteric ganglion of the cat

Blocking Agent	Stimulant	No. of Expts.	No. of Expts. in Which 50% or More Blockade Was Observed			
			40 μg	200 μg		1 mg
Morphine	DMPP	4	0	0		3
	5-HT		2	4		4
Cocaine	DMPP	5	1	2		5
	5-HT		4	4		5
BromoLSD	DMPP	5	1	4		5
	5-HT		4	5		5
3-Benzyloxygramine	DMPP	4	0	2		4
	5-HT		1	3		4
Dibenzyline	DMPP	4	0	0		2
	5-HT		0	0		4
Atropine	DMPP	5	2	5		5
	5-HT		0	3		5
			6 μg	25 μg	100 μg	400 μg
LSD	DMPP	4	0	0	0	3
	5-HT		0	0	1	4
Hexamethonium	DMPP	4		4	4	
	5-HT			0	0	

The selectivity and potency of morphine as an antagonist to 5-HT in the autonomic ganglia is marked. This suggests that certain agents, perhaps related to morphine, may furnish a new group of compounds which may be classified as ganglionic or, in general, neurotropic 5-HT antagonists.

SUMMARY

Evidence is obtained that 5-HT has a ganglionic stimulant action on the inferior mesenteric ganglion. The mechanism of its stimulant action is different from that of DMPP.

Morphine selectively blocks the ganglionic stimulant action of 5-HT. The selectivity is less marked with cocaine and bromoLSD.

Dibenzyline, benzyloxygramine and LSD do not show selectivity in blocking HT as compared with DMPP.

The *receptors* in the inferior mesenteric ganglion sensitive to HT are different from the cholinergic receptors. Some blocking agents known as potent antagonists to 5-HT on smooth muscle organs are relatively weak and not specific for the ganglionic 5-HT *receptors*.

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