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THE EFFECTS OF TRYPTAMINE ON THE CENTRAL NERVOUS SYSTEM, INCLUDING A PHARMACOLOGICAL PROCEDURE FOR THE EVALUATION OF IPRONIAZID-LIKE DRUGS

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One of the major problems which has complicated the study of the central nervous system effects of serotonin (5-hydroxytryptamine) is the relatively low level of penetration of this substance into the brain after systemic injection (Udenfriend *et al.*, 1957; Shore *et al.*, 1957). Udenfriend and co-workers (1957) attempted to overcome this problem by employing the precursor of serotonin, 5-hydroxytryptophane (5-HTP) to build up brain levels of serotonin. Although these workers demonstrated that systemic injection of 5-HTP did eventually raise brain serotonin levels, Bogdanski and co-workers (1958) reported that intraperitoneal injection of this substance into rats in massive doses (500 to [illegible] mg/kg) produced only mild effects.

[illegible] number of investigators have reported that [illegible]tamine acts at many of the same *peripheral* [illegible]ptor sites as does serotonin, but with less [illegible]nse effects (Gaddum, 1953; Woolley and Shaw, 1953[1]; Ginzel and Kottegoda, 1953; and many others). Accordingly, we proceeded to investigate the effects of tryptamine on the central nervous system, working on the hypothesis that tryptamine could also conceivably be effective in activating *brain* serotonin receptors. This report is concerned with a number of observations made on the effects of various drugs on the central effects of tryptamine. In addition, the significance of these observations with regard to the mechanism of action of serotonin is discussed.

[1] Woolley and Shaw (1957a) have also presented evidence which suggests that tryptamine and serotonin have separate receptors. Dogs given BAS failed to exhibit a pressor response after intravenous serotonin, whereas a pressor response to tryptamine was seen. These results may, however, be interpreted in another way. If tryptamine and serotonin do share the same receptor, it is conceivable that a substance such as BAS may block a portion of the receptor, for example the part which fits the hydroxyl group of serotonin, thus preventing the attachment by serotonin, whereas the receptor may remain responsive to tryptamine which has no hydroxyl group.

METHODS. Drugs were tested in adult male albino rats (Sprague-Dawley and Wistar strains), adult male and female albino rabbits, and adult male and female Macaca mulatta monkeys. The following drugs were investigated: tryptamine hydrochloride; 5-hydroxytryptamine creatinine sulfate (serotonin creatinine sulfate); *dl*-5-hydroxytryptophane (5-HTP); *d*-amphetamine sulfate (Dexedrine); *l*-ephedrine sulfate; pentylenetetrazol (Metrazol); strychnine sulfate; picrotoxin; methylphenidate hydrochloride (Ritalin); pipradrol hydrochloride (Meratran); β-phenylisopropyl-hydrazine (JB-516); iproniazid phosphate (Marsilid); caffeine; nikethamide (Coramine); mescaline sulfate; *d*-lysergic acid diethylamide (LSD-25); *d*-bromo-N, N-diethyllysergamide (BOL-148); chlorpromazine hydrochloride (Thorazine); trifluoperazine dihydrochloride (Stelazine); prochlorperazine dihydrochloride (Compazine); reserpine; phenobarbital sodium; diphenylhydantoin sodium (Dilantin); trimethadione (Tridione); 3-(2-aminoethyl)-1-benzyl-5-methoxy-2-methylindole hydrochloride (BAS); morphine sulfate; phenoxybenzamine hydrochloride (Dibenzyline); N-(3′-dimethylaminopropyl) iminodibenzyl hydrochloride (imipramine hydrochloride); benactazine hydrochloride (Suavitil); meprobamate, (Miltown); gamma aminobutyric acid (GABA). With the exception of reserpine, the drugs which were administered intravenously were solubilized in 0.9% saline solution. Reserpine was first solubilized in a vehicle containing citric and ascorbic acids, polyethylene glycol 400 and water. This solution was diluted to proper strength with saline.

RESULTS. The overt effects produced by the intravenous administration of tryptamine, serotonin, or 5-HTP in rats are summarized in table 1. In general, 8 to 10 rats were tested at each dose. Each of the agents tested produced similar

TABLE I

Overt effects of intravenous tryptamine, serotonin and 5-HTP in rats

Drug	Dose	Observations
	mg/kg i.v.	
Trypta-mine	1–40	Blanching of blood vessels of ears and paws followed by marked hyperemia
	2.5–40	Hypotonia, bradypnea, and exophthalmia
	10–40	Body tremors, bilateral placing-type clonic movements of forepaws, hunching of the back
	40	Backward locomotion, Straub tail, marked bradypnea and dyspnea, severe asymmetrical clonic convulsions, several rats also exhibited asphyxial convulsions and death
Serotonin	0.1–5	Blanching of blood vessels of ears and paws followed by marked hyperemia, bradypnea and dyspnea
	0.5–5	Hypotonia, inactivity
	1–5	Flaccid paralysis of hind limbs
	5	Marked cyanosis followed by severe asphyxial clonic convulsions and death
5-HTP	50	No overt effects
	100–400	Delayed (2 minutes after injection) onset of hypotonia, decreased locomotor activity, blanching of ears and paws followed by hyperemia
	400	Bradypnea and dyspnea

overt effects at the periphery, including blanching of the extremities followed by a sustained period of hyperemia. Tryptamine differed from the other compounds in that it caused bilateral placing-type clonic movements of the forepaws.

Various doses of tryptamine[2] were injected intravenously into groups of 8 or more rats and the number of animals responding at each dose with *3 seconds or more of uninterrupted clonic seizure activity was determined.* Depending on the dose administered, this seizure may vary from a 3-second period of unilateral or bilateral clonus of the forepaws to 30 or 40 seconds of clonus of both forepaws and hindpaws ending in a loss of balance. The per cent of animals exhibiting such convulsions was plotted (in probits) against the logarithm of dose and a straight line fitted to the points. The results of this experiment are illustrated in figure 1. From this dose-response curve 2 doses were selected, the convulsant dose-4 (CD4) and the convulsant dose-98 (CD98), *i.e.*, the doses of tryptamine which could be expected to cause convulsions in 4% and 98%, respectively, of animals tested. These doses were found to be

[2] Concentration — 2 mg/ml of saline.

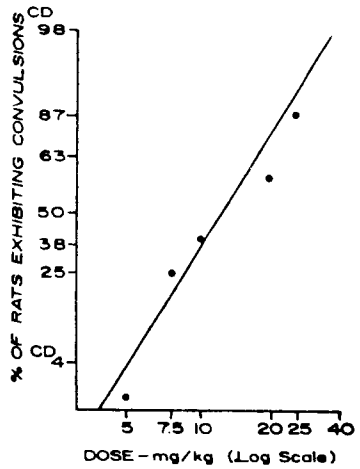

FIG. 1. Per cent of rats exhibiting 3 seconds or more of uninterrupted clonic seizure activity after intravenous injection of various doses of tryptamine.

The dose of tryptamine which would be expected to cause convulsions in 4% of rats (CD4) is 5 mg/kg; the dose which would be expected to cause convulsions in 98% of rats (CD98) is 40 mg/kg.

5 mg/kg and 40 mg/kg, respectively. In order to verify these doses, an additional 20 rats were injected with each dose: 0% responded with convulsions to the CD4, whereas 100% responded with convulsions to the CD98.

Investigation of drugs as tryptamine potentiators. All drugs were tested at their time of peak effect which was determined in the following manner. Five or more groups of 5 rats each were first treated with drug. Each group was injected intravenously with the CD4 of tryptamine at various time intervals thereafter, and the per cent of rats which responded with 3 seconds or more of uninterrupted clonic seizure activity determined. Drugs which caused seizures in 20% or more of rats were investigated further in the following manner. Three or more groups of 8 to 10 rats were treated with various doses of each drug and injected intravenously with the CD4 of tryptamine at the time of peak effect. In the case of iproniazid, for example, the time of peak effect was determined to be 18 hours after oral administration. Groups of 8 to 10 rats pretreated 18 hours earlier with oral doses of 5, 10, 25 and 50 mg/kg of iproniazid, exhibited a 10%, 50%, 100% and 100% incidence of convulsions, respectively, after injection with a CD4 of tryptamine. These percentages were plotted against

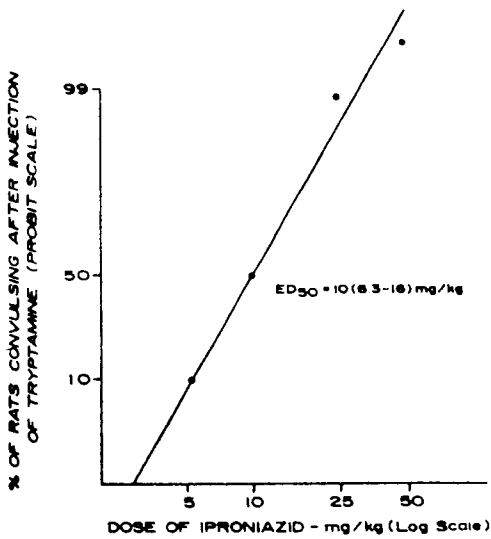

FIG. 2. Potentiating effect of various oral doses of iproniazid on tryptamine-induced convulsions in rats.

Iproniazid was administered orally 18 hours prior to the intravenous injection of a convulsant dose-4 (CD4) of tryptamine. The ED50 is defined as the dose of iproniazid effective in causing 50% of rats to respond with seizures after injection with the CD4 of tryptamine.

log-dose, as shown in figure 2.[3] The dose of iproniazid effective in causing 50% of rats to exhibit seizures after the intravenous injection of a CD4 of tryptamine (ED50) and 95% fiducial limits were determined by the method of Litchfield and Wilcoxon (1949). As shown in figure 2, the ED50 and 95% fiducial limits for iproniazid were found to be 10 (6.3—16.0) mg/kg.

In order to gain information about the mechanism by which iproniazid potentiated the convulsant effects of tryptamine, a large series of compounds possessing a variety of pharmacological actions was studied. A summary of the results obtained is presented in table 2. Although strychnine, pentylenetetrazol, picrotoxin, caffeine and nikethamide were each administered in doses which produced convulsions in some animals, intravenous injection of a CD4 of tryptamine failed to evoke convulsions in more than 28% of the remaining animals. Similarly, markedly stimulating doses of cocaine, *d*-amphetamine, methylphenidate, pipradrol or LSD-25 failed to potentiate the convulsant effects of tryptamine significantly. BOL-148 and BAS also failed to potentiate tryptamine. It is of interest that doses of ephedrine of 40 or 80 mg/kg produced a 50% potentiation of tryptamine, whereas doses greater than these produced progressively less potentiation. Drugs effective in potentiating the convulsant effects of tryptamine included iproniazid

[3] The 100% values were first appropriately corrected according to the method of Litchfield and Wilcoxon (1949).

TABLE 2

Effect of various drugs as tryptamine potentiators in rats

Drug	Route of Administration	Time of Peak Effect	Dose	% Potentiation
			mg/kg	
Strychnine sulfate	Oral	15 to 90 min	5	0
Pentylenetetrazol	Oral	15 min	150	25
Picrotoxin	Oral	15 to 180 min	15	0
Caffeine	Oral	3 hrs	300	12.5
Nikethamide	Oral	30 min	300	28
Cocaine HCl	Oral	15 to 180 min	120	0
d-Amphetamine sulfate	Oral	30 min	16	20
Methylphenidate HCl	Oral	15 min	60	10
Pipradrol HCl	Oral	3 hr	60	37.5
LSD-25	Oral	15 to 120 min	12	0
BOL-148	Subcutaneous	15 to 60 min	3	0
BAS	Oral	3 hr	100	0
Iproniazid	Oral	18 hr	ED50 = 10 (6.3-16.0) mg/kg	
B-Phenylisopropyl hydrazine (JB-516)	Oral	10 hr	0.5 1 2 4 ED50 = 1.1 (0.5-2.4) mg/kg	12.5 50 85.5 90
l-Ephedrine sulfate	Oral	30 min	5 10 20 40 80 120 180	0 16 16 50 50 33 0

and JB-516. JB-516 was found to be approximately 9 times as potent as iproniazid by this test procedure.

Tryptamine produced effects in the *monkey* which were qualitatively similar to those seen in rats. Intravenous doses of 10 mg/kg and above caused asymmetrical clonic convulsions followed by intermittent tremors of the body and extremities. The convulsant effects of tryptamine were quantitated in this species in a manner similar to that described for rats. It was determined by this procedure that an intravenous dose of tryptamine[4] of 5 mg/kg would be expected to induce convulsions in 1% of monkeys (CD1). In order to determine whether iproniazid was orally active in the monkey, a tryptamine potentiation experiment was performed similar to that described for rats. No attempt was made to determine the time of peak tryptamine potentiating activity of iproniazid, nor to quantitate its potentiating activity in this species. Each of 4 monkeys pretreated 5 hours previously with an oral dose of 100 mg/kg of iproniazid exhibited clonic convulsions after intravenous injection with the CD1 of tryptamine. This dose of iproniazid alone failed to produce any overt effects.

Investigation of drugs as antagonists of convulsant effects of tryptamine. The procedure employed in these investigations is similar to that described under tryptamine potentiators except that a CD98 of tryptamine[5] was employed and drugs were tested for their ability to prevent *5 seconds or more of uninterrupted clonic seizure activity.* The ED50 is defined as the dose of drug effective in preventing such seizures in 50% of rats.

Large oral doses of phenobarbital, diphenylhydantoin, trimethadione, meprobamate, reserpine, GABA, phenoxybenzamine, benactazine, mescaline, 5-HTP, serotonin, BAS, morphine, or imipramine failed to antagonize the convulsant effects of tryptamine. Compounds effective in antagonizing tryptamine included chlorpromazine, prochlorperazine, trifluoperazine, LSD-25 and BOL-148. Although rats pretreated with these compounds failed to exhibit characteristic clonic seizure activity, their behavior after injection of tryptamine was quite unusual. For example, rats pretreated with protective doses of chlorpromazine, prochlorperazine, or trifluoperazine, and then injected with the CD98 of tryptamine sat up on their hind legs and remained immobile for a period of approximately 1 minute. During this time they exhibited tremors of the head and body and pronounced hyperemia of the ears and paws. Rats injected with protective doses of LSD-25 or BOL-148 and then injected with a CD98 of tryptamine exhibited backward locomotion, side-to-side head movements and occasional head and body tremors.

ED50's and 95% fiducial limits for these compounds as tryptamine antagonists are presented in table 3. Statistical analysis of the data revealed that the 3 phenothiazines tested, namely chlorpromazine, prochlorperazine and trifluoperazine, did not differ significantly from one another in tryptamine antagonist potency ($P > .05$). Similarly, LSD-25 and BOL-148 were found to be equivalent to each other in tryptamine antagonist potency both orally as well as subcutaneously. Doses of BOL-148 effective in antagonizing tryptamine failed to produce any overt effects. In contrast, doses of LSD-25 effective in antagonizing tryptamine also caused piloerection, increased locomotor activity, disorientation, head and body tremors, etc.

Antagonism of the convulsant effects induced by 5-HTP in rats pretreated with iproniazid. In view of the findings of Bogdanski and co-workers (1958) that pretreatment of rats with iproniazid markedly potentiates the stimulant effects of 5-HTP, several rats were treated with an oral dose of 50 mg/kg of iproniazid and 20 hours later injected intravenously with a dose of 50 mg/kg of 5-HTP. The following effects were observed. The rats at first exhibited a marked increase in locomotor activity and curiosity. This was followed, in 3 to 5 minutes, by shaking movements of the head and an asymmetrical placing-type clonus of the forepaws. The clonus lasted for a minute or longer and was followed by a sustained episode of body tremors. The clonic seizures observed were strikingly similar to those seen in rats injected with convulsant doses of tryptamine. The convulsant effects of 5-HTP in iproniazid pretreated animals were quantitated in the following manner. Thirty rats were treated orally with a dose of 50 mg/kg of iproniazid and divided into 3 groups of 10 rats each. Twenty hours later these groups of rats were injected intravenously with doses of 25, 35 and 50 mg/kg, respectively, of 5-HTP. The end point for a convulsion was taken as 5 seconds or more of *uninterrupted* clonic

[4] Concentration — 20 mg/ml of water.
[5] Concentration — 8 mg/ml of water.

seizure activity occurring during the 15-minute period following the injection of 5-HTP. These doses of 5-HTP produced an incidence of convulsions of 0, 62.5 and 87.5%, respectively. These doses and percentage responses were plotted on log-probability paper and the dose of 5-HTP which could be expected to effect convulsions in 98% of rats pretreated with 50 mg/kg of iproniazid (CD98) determined to be 55 mg/kg.

BOL-148 was then tested for its ability to prevent convulsions induced in rats pretreated with iproniazid and later injected with 5-HTP. Ten rats were treated orally with iproniazid, 50 mg/kg, and 20 hours later injected subcutaneously with 3 mg/kg of BOL-148. Thirty minutes after the injection of BOL-148 (time of peak effect) the rats were injected intravenously with the CD98 of 5-HTP. Five of the 10 rats treated in this manner failed to exhibit convulsions, which indicates that BOL-148 was effective in preventing seizures induced by the combination of iproniazid followed by 5-HTP.

Reversal of reserpine-induced depression by tryptamine infusion in rabbits. A summary of the effects of an intravenous infusion of tryptamine alone as well as in combination with iproniazid and/or reserpine in rabbits is presented in table 4. Groups of 4 or more rabbits were treated with each drug or combination of drugs. The observations summarized in the table demonstrated that tryptamine produced effects in rabbits qualitatively similar to those seen in rats. Of considerable significance was the fact that tryptamine was extremely effective in reversing reserpine-induced depression in this species, although the effects were of short duration. When, however, tryptamine was infused into reserpinized rabbits which had been later treated with iproniazid, the reserpine-reversing action of tryptamine was both markedly potentiated and prolonged.

DISCUSSION. The intravenous injection of either serotonin or tryptamine into rats resulted in similar overt peripheral effects, characterized by initial blanching of the ears and paws, followed by a sustained period of hyperemia. This similarity of effect may be attributed to activation of similar peripheral receptors as originally postulated by Gaddum (1953), Woolley and Shaw (1953), and Ginzel and Kottegoda (1953).

Although both tryptamine and serotonin produced convulsions in rats, serotonin-induced convulsions were obviously asphyxial in origin, since they occurred only after a sustained period of

TABLE 3

Activity of various compounds in antagonizing tryptamine convulsions in rats

Drug	Route of Administration	Time of Peak Effect	Dose	% Protected
			mg/kg	
Phenobarbital	Oral	1 to 3 hr	100	0
Diphenylhydantoin	Oral	½ to 3 hr	100	0
Trimethadione	Oral	1 to 3 hr	500	0
Meprobamate	Oral	½ to 3 hr	300	0
Reserpine	Oral	1 to 24 hr	8 and 50	0
Chlorpromazine	Oral	3 hr	ED50 = 9 (5.3–15.3) mg/kg	
Prochlorperazine	Oral	4½ hr	ED50 = 11.2 (8.5–15) mg/kg	
Trifluoperazine	Oral	3 hr	ED50 = 14 (11.7–16) mg/kg	
GABA	Oral	1 to 3 hr	400	0
Phenoxybenzamine HCl	Oral	3 hr	400	0
Benactazine HCl	Oral	3 hr	10	0
Mescaline HCl	Oral	3 hr	200	0
5-HTP	Oral	3 hr	400	0
Serotonin creatinine sulfate	Oral	1 to 16 hr	400	0
BAS	Oral	2 hr	400	0
Morphine sulfate	Oral	½ to 3 hr	200	0
Imipramine HCl	Oral	3 hr	100	0
LSD-25	Oral	30 min	ED50 = 25 (22–28) mg/kg	
LSD-25	Subcutaneous	15 min	ED50 = 1.4(0.9–2.3) mg/kg	
BOL-148	Oral	30 min	ED50 = 23 (18–30) mg/kg	
BOL-148	Subcutaneous	30 min	ED50 = 1.2(0.7–2.2) mg/kg	

TABLE 4

Effects of tryptamine alone and in combination with iproniazid on reserpine-induced depression of rabbits

Drug(s) Administered	Dose	Observations
	mg/kg i.v.	
Tryptamine (infusion)	1.5 mg/kg/min	Mydriasis, decreased curiosity and locomotor activity
	2–3 mg/kg/min	Clonic convulsive movements of forepaws after 5 to 10 min
Reserpine	5	Prostration, marked ptosis and miosis, occasional spontaneous clonic convulsive movements
Reserpine Tryptamine (infused 3 to 4 hr after reserpine administration)	5 1.5 mg/kg/min	After 1 to 2 min of tryptamine infusion, the reserpine induced prostration; ptosis and miosis were reversed; rabbits again exhibited normal exploratory behavior. They remained alert as long as the infusion was continued. Approximately 2 min after the infusion was stopped, the characteristic reserpine depression again became manifest (ptosis, miosis, prostration, etc.)
Iproniazid	50	Slight to moderate mydriasis
Iproniazid Tryptamine (infused 6 to 8 hr after administration of iproniazid)	50 0.1 mg/kg/min	Twenty sec after the tryptamine infusion was begun, the rabbits exhibited a slight increase in exploratory behavior and brief intermittent pushing movements of the forepaws. Increasing the dose of tryptamine to 0.2 mg/kg/min exaggerated these effects.
Reserpine Iproniazid (administered 4 hr after reserpine)	5 50	Prostration, marked ptosis, miosis, occasional spontaneous convulsive movements
Reserpine Iproniazid (administered 4 hr after reserpine) Tryptamine (infused 4 hr after administration of iproniazid)	5 50 0.1 mg/kg/min	Approximately 20 sec after the infusion was begun, the reserpine depression was reversed. Animals assumed a normal posture, ptosis and miosis were no longer present. When the tryptamine infusion was stopped shortly after arousal, the *rabbits remained alert for* ½ hr or longer. When, however, the tryptamine infusion was continued after arousal, the rabbits went on to exhibit typical tryptamine convulsions
Iproniazid Reserpine (administered 4 hr after iproniazid)	50 5	Rabbits treated in this manner failed to exhibit the same degree of reserpine depression as caused by this dose of reserpine alone.

dyspnea followed by apnea, and were always accompanied by distinct cyanosis. Udenfriend and co-workers (1957) have reported that systemically injected serotonin does not readily penetrate into the brain which may explain why serotonin failed to produce direct overt central effects. Tryptamine-induced convulsions, in contrast, did appear to be central in origin. Evidence favoring this view was the fact that no signs of respiratory embarrassment such as dyspnea or

cyanosis were present prior to their onset. In addition, phenoxybenzamine which has been demonstrated to antagonize effectively several peripheral actions of tryptamine, including contractile effects of tryptamine on the rat uterus and pressor effects of tryptamine in dogs (D. Tedeschi, unpublished), failed to antagonize the convulsant effects of tryptamine. This provides a further indication of a central mechanism of action for tryptamine.

Since serotonin did not readily penetrate into the central nervous system, Udenfriend and coworkers (1957) employed 5-HTP to increase serotonin brain levels. These investigators demonstrated that 5-HTP readily penetrated into the brain, after systemic injection, where it was rapidly decarboxylated by 5-HTP decarboxylase to form serotonin. In addition, they reported that pretreatment of rats with iproniazid followed by the injection of 5-HTP markedly increased brain serotonin levels. In view of these findings, it is suggested that the convulsions observed in rats pretreated with iproniazid and later injected intravenously with 5-HTP may be attributable to increased brain serotonin levels. This explanation could account for the observed delay in onset of convulsions (3 to 5 minutes) seen in rats treated in this manner. This delay presumably reflects the time required for decarboxylation of 5-HTP to serotonin in sufficient quantity to reach a convulsant level in the brain. Although the 5-HTP-iproniazid combination was quite effective in inducing convulsions in rats, massive doses of 5-HTP alone were ineffective. This observation suggests that the time required for the conversion of 5-HTP to serotonin is equal to or greater than the time required for the destruction of serotonin by monoamine oxidase. Consequently, in the absence of a monoamine oxidase inhibitor, brain levels of serotonin sufficient to cause convulsions may not be reached. When 5-HTP is injected into rats pretreated with a monoamine oxidase inhibitor (iproniazid), the destruction of serotonin by this enzyme is inhibited, thus permitting sufficient concentrations of serotonin to accumulate to induce convulsions.

Assuming that increased brain serotonin levels are responsible for seizures induced by the combination of 5-HTP and iproniazid, it may then be postulated that tryptamine and serotonin activate similar brain receptors. Evidence favoring this hypothesis is the striking similarity in seizures induced by either tryptamine or 5-HTP in combination with iproniazid. In addition, BOL-148 was found to be an effective antagonist of convulsions induced by either of these procedures. Experiments performed with reserpine also support this hypothesis. The failure of reserpine to antagonize tryptamine-induced convulsions suggests that reserpine does not block brain receptors activated by tryptamine. Tryptamine, however, *was* effective in reversing reserpine-induced depression. Shore and co-workers (1957) have reported that reserpine causes a pronounced depletion of brain serotonin levels, which may serve as a possible explanation for the effects observed in the present experiments. Under these circumstances, reserpine would not be expected to antagonize tryptamine, whereas, if tryptamine did indeed activate brain serotonin receptors it *would* be expected that tryptamine would reverse effects resultant from brain serotonin depletion, namely reserpine-induced depression. (Other mechanisms by which reserpine may act to produce its depressant effects such as depletion of brain catecholamines must, of course, continue to be considered.) The transient nature of the reserpine-reversal effected by tryptamine may be attributed to the rapid destruction of tryptamine by monoamine oxidase, since in the presence of iproniazid, tryptamine effected a sustained reversal of reserpine-induced depression.

Three of the many possible mechanisms by which a drug might act to potentiate the convulsant effects of tryptamine are: a) by preventing the inactivation of tryptamine through inhibition of the enzyme system responsible for its destruction, *i.e.*, monoamine oxidase; b) by subliminal stimulation of the same receptor sites that are activated by tryptamine; and c) by stimulating other parts of the nervous system, thus lowering the threshold for tryptamine stimulation.

Both of the compounds demonstrated to be potent tryptamine potentiators, namely, iproniazid and JB-516, have also been demonstrated to be potent *in vivo* inhibitors of monoamine oxidase (iproniazid, Zeller and Barsky, 1952, and JB-516, Horita, 1958). Since Schayer and coworkers (1954) reported that tryptamine is metabolized almost entirely by monoamine oxidase, this may be taken as presumptive evidence that these drugs may be potentiating tryptamine by preventing its destruction. The possibility

nevertheless exists that these drugs might also be acting by one of the other mechanisms described above. In order to explore mechanisms b) and c) a number of central nervous system stimulants were investigated. It was quite surprising that barely subconvulsant doses of pentylenetetrazol, picrotoxin, strychnine, caffeine, and nikethamide, failed to potentiate tryptamine convulsions. In addition, doses of *d*-amphetamine, pipradrol, cocaine, methylphenidate, and LSD-25 which in themselves produced marked CNS stimulation also failed to potentiate tryptamine. The possibility exists that these stimulants influence parts of the CNS which act independently from areas stimulated by tryptamine, and therefore fail to act jointly with tryptamine.

The results obtained after oral administration of ephedrine were quite unexpected. Although Gaddum and Kwiatkowski (1938) proposed that one of the possible mechanisms by which ephedrine potentiates the vasoconstrictor response to epinephrine was by monoamine oxidase inhibition, several objections to this hypothesis have been raised (Richter and Tingey, 1939; Jang, 1940). More recently, Schayer and co-workers (1954) reported that ephedrine was a poor monoamine oxidase inhibitor in intact animals. The results of the present investigation support this conclusion, since markedly stimulating doses of ephedrine were required to produce a 50% potentiation of tryptamine. The explanation for the progressive decrease in incidence of convulsions with increasing dosage above 80 mg/kg remains obscure, however.

Unfortunately, biochemical techniques are not useful for demonstrating whether a drug prevents serotonin from activating its brain receptor sites, since in this situation changes in the concentration of brain serotonin or its metabolite would not be expected to occur. Although LSD-25 has been postulated to act by antagonizing brain serotonin, the evidence favoring this hypothesis has been, of necessity, indirect. For example, Gaddum (1953) made this proposal on the basis of the antagonistic effects of LSD-25 against serotonin on the rat uterus. Shore and co-workers (1955) potentiated hexobarbital with reserpine and postulated that the mechanism of this potentiation involved release of serotonin by reserpine. LSD-25 reversal of the reserpine-induced potentiation was attributed to antagonism of the released serotonin by the LSD-25. Woolley and Shaw (1957b) produced behavioral changes in mice with LSD-25 and then reversed these by injecting serotonin directly into the brain. These workers felt that this was evidence of interference by serotonin in mental disturbances resulting from administration of LSD-25.

If the hypothesis is accepted that tryptamine produces its stimulant effects by activation of serotonin receptors in the brain, prevention of tryptamine convulsions should provide an excellent means for testing agents which prevent the activation of brain serotonin receptors. Working under this assumption, a large number of compounds encompassing a broad spectrum of pharmacologic activity was investigated. Three anticonvulsant drugs were tested to determine whether such compounds would antagonize tryptamine convulsions, possibly by a nonspecific anticonvulsant action. The failure of large doses of phenobarbital, diphenylhydantoin, or trimethadione to prevent the tryptamine convulsions provides evidence which favors a selective type of stimulation, since the doses of phenobarbital employed are known to prevent convulsions induced by a variety of convulsant agents. The failure of BAS to block the convulsant effects of tryptamine may possibly be attributed to the failure of this substance to penetrate into the brain. Indirect evidence favoring this view has been reported by Woolley and Shaw (1957b). However, the possibility also exists that compounds which block the peripheral effects of serotonin may not necessarily block the central effects of tryptamine and *vice versa* (D. Tedeschi, unpublished).

It was of considerable interest that chlorpromazine, prochlorperazine and trifluoperazine prevented tryptamine convulsions. A number of investigators have reported that chlorpromazine is a serotonin antagonist when tested for this effect at peripheral receptor sites (Gyermek, 1955; Costa, 1956). In addition, it has been observed (D. Tedeschi, unpublished) that chlorpromazine produces an insurmountable and essentially irreversible blockade of the contractile effects of serotonin on the rat uterus. It is, therefore, not unreasonable to speculate that a similar type of antagonistic action may occur in the central nervous system.

There appears to be a poor correlation between the effectiveness of phenothiazines as tryptamine antagonists and their effectiveness in the condi-

tioned response test. For example, although chlorpromazine has been reported to be only half as potent as prochlorperazine as a conditioned response blocker (Cook and Weidley, 1957; Cook, 1958), it was found to be approximately equivalent in potency to prochlorperazine as a tryptamine antagonist. Trifluoperazine, which has been reported to be almost 10 times as potent as chlorpromazine as a conditioned response blocker (Tedeschi *et al.*, 1959) was also found to be equal in potency to chlorpromazine as a tryptamine antagonist. Because of the relative lack of tryptamine antagonist activity seen in rats treated with trifluoperazine, it may be concluded that this component of its pharmacologic action probably contributes very little toward its over-all effectiveness as a tranquilizer.

The significance of central tryptamine or serotonin antagonist activity with regard to the mode of action of psychotherapeutic agents is not clearly understood. As shown in table 3, the great majority of drugs tested, including phenobarbital, meprobamate, reserpine, benactazine, morphine, imipramine, etc., were devoid of significant tryptamine antagonist properties.

The finding of central tryptamine antagonist activity with LSD-25 supports Gaddum's proposal (1954) that this drug could conceivably block brain serotonin receptors. The data presented suggest, however, that this action of LSD-25 occurs independently of its psychotomimetic effects, since BOL-148, an agent which apparently does not produce hallucinogenic effects in man (Snow *et al.*, 1955) was nevertheless quite effective as a tryptamine antagonist. In this regard, it was of interest that doses of LSD-25 effective in antagonizing tryptamine also caused pronounced overt effects such as piloerection, tremors, etc., prior to the injection of tryptamine. Effective tryptamine antagonist doses of BOL-148, in contrast, failed to produce overt effects. This observation suggests that LSD-25 is capable of producing a number of additional pharmacological actions which may occur quite independently of its tryptamine and/or serotonin antagonist properties.

The effects observed with tryptamine in the present studies have been explained on the assumption that tryptamine acts at the same receptor sites as serotonin in the brain, which may indeed be the case. The recent finding by Hess and and co-workers (1959) of tryptamine in the central nervous system suggests the possibility that tryptamine may also act quite independently of serotonin. In any case, biochemical determinations of the concentration of tryptamine present in brain after administration of reserpine, iproniazid, etc., should serve to elucidate further the role of tryptamine in brain function.

SUMMARY

A pharmacological procedure is described by which drugs are tested for their ability to potentiate or antagonize convulsions induced by the intravenous injection of tryptamine. Drugs effective in potentiating convulsant effects of tryptamine included iproniazid and JB-516. Drugs ineffective as tryptamine potentiators included strychnine, pentylenetetrazol, picrotoxin, caffeine, nikethamide, cocaine, *d*-amphetamine, methylphenidate, pipradrol, LSD-25 and BOL-148. Ephedrine produced significant potentiation only in a relatively narrow range of dosage.

LSD-25, BOL-148, chlorpromazine, prochlorperazine and trifluoperazine were demonstrated to be effective in antagonizing the convulsant effects of tryptamine. In contrast, phenobarbital, diphenylhydantoin, trimethadione, meprobamate, reserpine, GABA, phenoxybenzamine, benactazine, mescaline, 5-hydroxytryptophane, serotonin, BAS, morphine and imipramine failed to antagonize tryptamine convulsions.

An infusion of tryptamine into reserpinized rabbits effectively reversed the ptosis, miosis and depression induced by reserpine. This reserpine-reversing action of tryptamine was markedly potentiated and prolonged by combination with iproniazid. The significance of these findings in relation to the mechanisms of action of the drugs tested is discussed.

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