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THE SIGNIFICANCE OF METHYL GROUPS IN THE ELECTROENCEPHALOGRAPHIC EFFECTS OF INDOLEALKYLAMINES IN THE RABBIT

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Abstract—The effects of tryptamine, 5-methoxytryptamine, *N*-dimethyltryptamine, and 5-methoxy-*N*-dimethyltryptamine on the central nervous system of rabbits were studied in order to determine their sites of evoking EEG arousal patterns. In animals with intact brain all the compounds, with the exception of tryptamine, induced well-sustained EEG arousal patterns. Transections both immediately rostral and caudal to the midbrain revealed that the non-psychotomimetic 5-methoxytryptamine induced the EEG arousal pattern in the midbrain. But for the psychotomimetics dimethyltryptamine and 5-methoxy-*N*-dimethyltryptamine, a site for evoking the EEG arousal pattern was found in the medullary area.

IN OUR previous papers[1, 9] we noted that phenylethylamines with two or three methoxy groups, such as mescaline and 3,4-dimethoxyphenylethylamine, produced electroencephalographic (EEG) arousal patterns at the medullary level in common with the psychotomimetic indolamines LSD, bufotenin, and psilocybin.[2–4] But non-*O*-methylated phenylethylamines, such as epinephrine and amphetamine, as well as 4-methoxyphenylethylamine with one methoxy group, provoked the EEG arousal pattern at a higher level of the neuraxis in the midbrain.

Our interest in *O*-methylation of indolealkylamines was promoted by the finding that melatonin (*O*-methylated *N*-acetylserotonin) is formed from serotonin in the pineal body.[5] McIsaac[6] demonstrated that one of the proposed metabolites of melatonin, 10-methoxyharmalan, is structurally related to the psychotomimetic alkaloids harmine and harmaline, and induces autonomic changes in addition to disorganizing conditioned behavior in experimental animals. Furthermore, Gessner *et al.*[7] pointed out that *O*-methylated bufotenin (5-methoxy-*N*-dimethyltryptamine)[8] was more potent than the parent compound without the methoxy group in disrupting trained animal behavior. Thus, *O*-methylation of indolealkylamines also may play an important role in central nervous mechanisms.

The present experiments were performed in order to compare the sites of EEG arousal induced by tryptamine and *N*-dimethyltryptamine with those of their *O*-methylated derivatives and to determine whether or not the introduction of methoxy groups shifts the site for evoking EEG alerting from the midbrain to the medullary region. In our previous studies we observed that the introduction of methoxy groups

into phenylethylamine did influence a shift in the site of alerting to the medullary region.[1, 9]

METHODS AND MATERIALS

Seventy-five adult New Zealand albino rabbits ranging in weight from 2·5 to 2·9 kg were used in these experiments. The animals were immobilized with injections of curare and artificially respired after tracheotomy under ether anesthesia. For further surgical interference, only local Pontocain (tetracaine) anesthesia was used. Monopolar electrodes were implanted according to the charts of Sawyer *et al.*[10] on the anterior (motor) and posterior (limbic) cortical areas and also in the head of the caudate nucleus, thalamus, dorsal hippocampus, and amygdala for EEG recording. Electroencephalogram, electrocardiogram (EEG), and arterial blood pressure were registered by means of an 8-channel Grass model III-D electroencephalograph. For recording of arterial blood pressure, a polyethylene cannula connected to a Statham P23-G transducer was inserted into a femoral artery.

In order to diminish reflex influences the carotid sinus and carotid body were denervated bilaterally (by stripping the tissues around the bifurcations of the internal carotid and occipital arteries from the common and external carotid arteries with a forceps) and by the application of a cotton ball soaked with 10% formalin, in addition to bilateral vagotomy. In this communication animals so prepared will be referred to as denervated preparations for the sake of brevity.

Transections of the brain were performed at three levels: prepontine, precollicular just above the midbrain; postcollicular, postpontine just below the midbrain; and through the first segment of the cervical spinal cord. For transection at the precollicular, prepontine level, the brain tissue was cauterized with a Wappler cold cautery scalpel inserted through three or four holes made on each side of the sagittal suture and in a parallel line, 11 mm posterior to the coronal suture. The holes were then connected with a rongeur in order to sever the brain stem entirely by transection with a blunt spatula.

Transection of the brain at the postcollicular, postpontine level was performed by passing a blunt spatula into the brain through the windows made on a line each side of the sagittal suture parallel and 22 mm posterior to the coronal suture. Only the transected preparations in which the mean blood pressure levels were appreciably unchanged from those prior to transection were used for injection of the agents tested.

The transections at the first cervical level (C1) were made with an excavator under direct view, after the atlanto-occipital membrane had been removed. A single dose (0·2 mg/kg) of ephedrine was injected i.v. just before the transection to protect against the hypotension induced by spinal shock, and only those preparations exhibiting a stable blood pressure level of more than 55 mm Hg were used for our experiments. All transections were examined postmortem to confirm the completeness of the transection. The locations of implanted electrodes were examined histologically in some preparations, including those with intact brain.

In most animals the various agents were injected through a polyethylene cannula inserted in a femoral vein. Some animals received continuous i.v. infusions at a constant rate with an infusion pump. In others, intracarotid injection was given through

a T-cannula inserted into a common carotid artery. The following agents were used: tryptamine hydrochloride, 5-methoxytryptamine (base), *N*-dimethyltryptamine oxalate, and 5-methoxy-*N*-dimethyltryptamine oxalate. Chemical structures are shown in Fig. 1. 5-Methoxytryptamine was dissolved in 0·05 N HCl solution

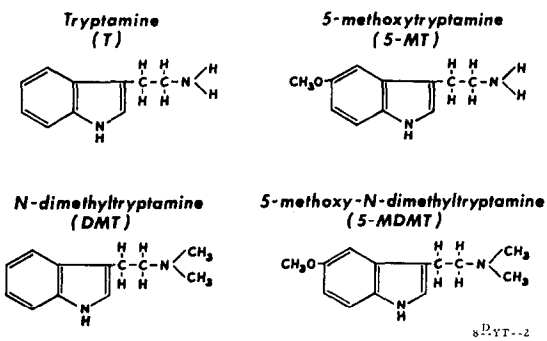

FIG. 1. Chemical structures of tryptamine derivatives used in present experiments.

and its pH adjusted to 7·0 with sodium bicarbonate. Other agents were dissolved in distilled water.

RESULTS

Control EEG patterns in rabbits before and after peripheral stimulation

In about 90 per cent of EEG patterns recorded without any agents or external stimulation, the rabbits with intact brain showed EEG resting patterns of high amplitude slow waves, sometimes with superimposed spindles. Acoustic and tactile stimulation were evoked by hand-clapping and pinching the footpad respectively. These EEG changes were characterized in both cortices by low amplitude fast waves and in the hoppocampus by regular theta waves (Figs. 2 and 4). These control patterns lasted for 10–20 sec.

Types of EEG alerting in drug-injected animals

After the administration of various agents, EEG patterns indistinguishable from the control patterns were referred to simply as EEG arousal patterns. In some animals, however, these drug-evoked EEG patterns demonstrated, in addition to the low amplitude fast waves characteristic of alerting, some degree of spindling in the traces from the motor cortex, caudate nucleus, and thalamus, and these were accompanied by waxing and waning waves in the limbic cortex, as well as irregular waves in the hippocampus. Such EEG changes were referred to as the modified EEG arousal pattern.

Tryptamine

Tryptamine was injected via vein or via the carotid artery at dosages of 0·01–1 mg/kg to seven rabbits with intact brain. This agent was administered in concentrations of 0·01–0·03 mg/kg by continuous i.v. infusion in three other rabbits. Although a total dose of 4 mg/kg was given by either route, a very brief arousal,

associated with a rise in blood pressure, was sometimes obtained, but in most animals the EEG changes occurred only in association with decreases in blood pressure and cardiac arrhythmias (Table 1).

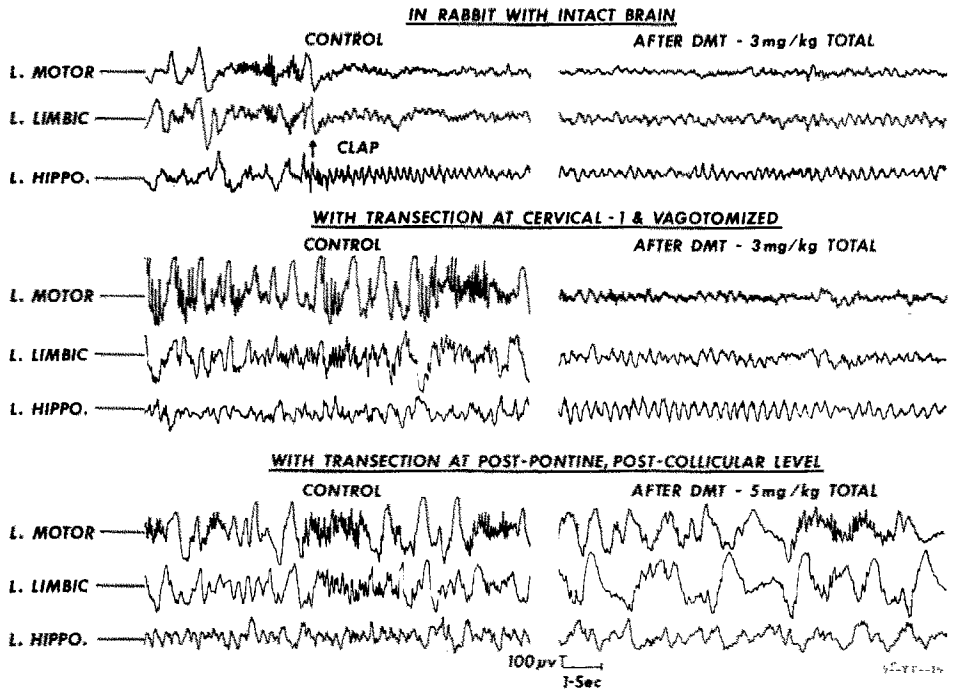

FIG. 2. EEG effects of *N*-dimethyltryptamine in rabbits. Control on left side. On right side note EEG arousal patterns in animals with intact brain and after transection at C1 plus denervation. But EEG arousal patterns were blocked by previous transection caudad to the midbrain.

5-Methoxytryptamine (5-MT)

Typical EEG arousal patterns (Fig. 3) were observed for 1–3 min after i.v. injections of 0·2 Mo 0·5 mg 5-MT/kg in 4 of 13 rabbits with intact brain. In the other nine animals the injection of 5-MT at the dosage level of 1 mg/kg produced typical EEG arousal patterns which were sustained for about 10 sec and were accompanied by moderately decreased amplitudes in the hippocampus for the next 1 min thereafter. During the following 5 min or so, modified EEG arousal was observed before patterns returned to the preinjection character. No cumulative effect was seen even after a total dosage of 7 mg/kg was attained by repeated injections at 7-min intervals. Mean levels of arterial blood pressure were elevated by 5–15 mm Hg for approximately 2 min after each injection. After postcollicular, postpontine transections, 1 mg 5-MT/kg produced EEG arousal patterns for the same period as, or longer than, the control of each of five preparations. In four precollicular, prepontine transected preparations, after 1 mg 5-MT/kg, only one exhibited a brief EEG arousal pattern, and all the remainder failed to show EEG changes. These results are summarized in Table 1.

N-*Dimethyltryptamine* (DMT)

This agent was injected i.v. at 7–10-min intervals at dosages of 0·5, 1·0 or 2·0 mg/kg (Fig. 2; Table 1). In four rabbits with intact brain and a dose of 0·5 mg/kg, each injection was immediately followed by typical EEG arousals persisitng for approximately 40 sec, but these changes sometimes failed to appear as the dose was repeated.

TABLE 1. EEG EFFECTS OF TRYPTAMINE DERIVATIVES IN VARIOUS PREPARATIONS

Drug	Total dosage level (mg/kg)	Preparation	Site of transection	Total no. of animals used	No. of animals exhibiting EEG effects* after drug	Level for EEG arousal
Trypt.	4·0	Intact		10	0	
5-MT	1·0	Intact		9	9	Mesencephalic
	1·0	Transection	Postcollicular, postpontine	5	5	
	1·0	Transection	Precollicular, prepontine	4	1	
DMT	2·0–5·0	Intact		10	6	
	2·0	Denervated		3	3†	Medullary‡
	3·0–5·0	Transection without vagotomy	First cervical vertebra	4	1	
	3·0–5·0	Transection with vagotomy	First cervical vertebra	4	3	
	4·0–5·0	Transection	Postcollicular, postpontine	5	0	
5-MDMT	0·1–0·3	Intact		9	9	Medullary‡
	0·1–0·2	Denervated		3	3†	
	0·1–0·25	Transection with vagotomy	First cervical vertebra	4	4	
	0·4–0·5	Transection	Postcollicular, postpontine	5	0	

* Typical or modified EEG arousal.

† Denervation of carotid body and carotid sinus intensifies EEG arousal.

‡ EEG arousal facilitated by spinal afferent input.

A total of 4 mg/kg (repeated injections of 0·5 mg/kg) produced typical EEG arousal for about 20 min in one of four rabbits and modified arousal was maintained for 3 min in another animal. These EEG changes appeared about 5 min after the final injection. The other two animals failed to show EEG changes except for brief initial EEG arousal patterns.

A dose of 1 mg/kg for each injection was used in another six rabbits with intact brain. Each injection was followed by typical EEG arousal patterns in the same manner as those seen after 0·5 mg/kg. After a total dosage of 2–5 mg/kg, typical EEG arousal patterns were sustained for periods of approximately 25 min or for 5 min after a series of 4–10 injections, in two preparations. In the other two animals given a total dosage of 3 and 5 mg/kg, modified EEG arousals occurred 5 and 7 min respectively after the final injection, and this response endured for 10–30 min. Although a total of 5 mg/kg was injected in the two remaining animals, both exhibited only slight decreases of slow waves and spindles. In the last two animals each injection at a level of 0·5 or 1·0 mg/kg was immediately followed by a moderate rise in

blood pressure as well as bradycardia for nearly 2 min. These cardiovascular effects tended to be smaller as dosage was repeated. In general, single injections at a dose of 2 mg/kg produced toxic effects including decreases in blood pressure and cardiac arrhythmias. In two rabbits with remarkable bradycardias after short-lasting pressor

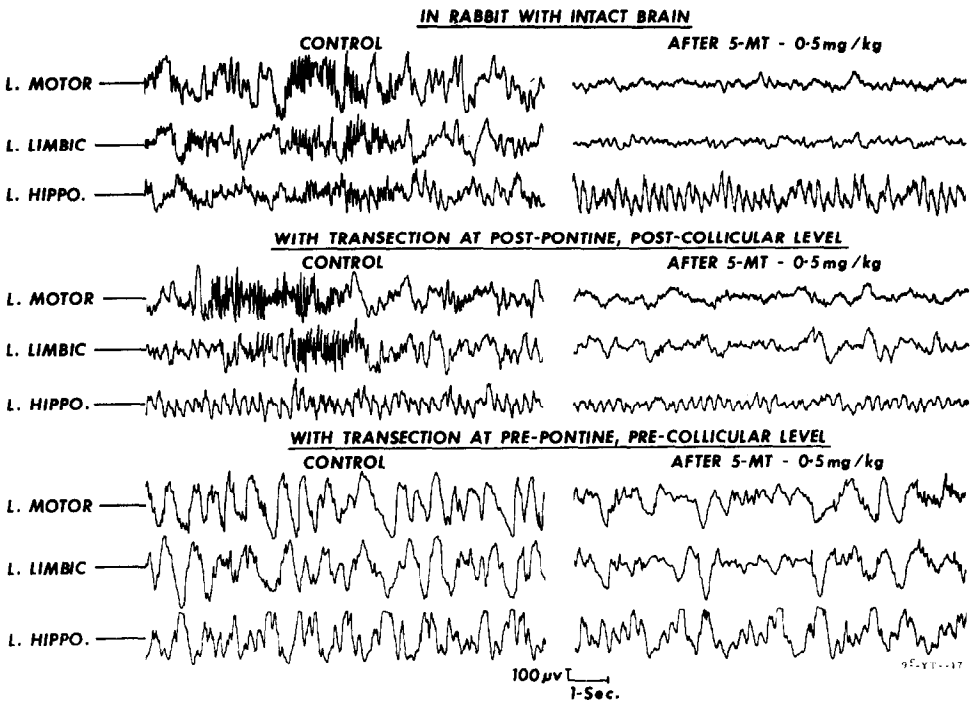

FIG. 3. EEG effects of 5-methoxytryptamine (5-MT) in rabbits. Control on left side. On right side note EEG arousal patterns in rabbits either with intact brain or after transection just below the midbrain. In the precollicular, prepontine transected preparation, however, an EEG arousal pattern did not occur.

response, delta waves were seen in all leads. In the third, modified EEG alerting was observed for about 20 min. These results indicated that injections of DMT at dosage levels of 1 mg/kg at 7–10-min intervals were adequate for study in the following experiments.

In one of three preparations with bilateral denervation of the carotid sinus and carotid body plus vagotomy, a total dosage of 2 mg/kg produced typical EEG arousal patterns immediately after injection; this pattern continued for about 20 min. In the other two preparations this total dose provoked two phases of EEG arousal patterns consisting of immediate short-lasting ones coincident with rises in blood pressure and delayed continuous ones which appeared 5–7 min after the final injection. Bradycardia observed in the control was almost completely eliminated in these two preparations.

In one of four animals with intact vagal nerves and transection at C1, modified EEG arousal was obtained after a total dosage of 3 mg/kg, but in the other three animals no notable EEG changes were induced even after a total of 5 mg/kg. A total

dose of 3–5 mg/kg produced either typical or modified EEG arousal patterns which were maintained for about 10 min in three of four preparations previously transected at C1 and in addition subjected to bilateral vagotomy. Transections at postcollicular, postpontine level prevented the development of EEG arousals even after total dosages of 4–5 mg DMT/kg in each of the five preparations.

5-*Methoxy*-N-*dimethyltryptamine* (5-*MDMT*)

Ten rabbits with intact brain were used to observe effects on EEG of this agent given i.v. at 10-min intervals in dosages of 0·025–0·5 mg/kg (Fig. 4; Table 1). A

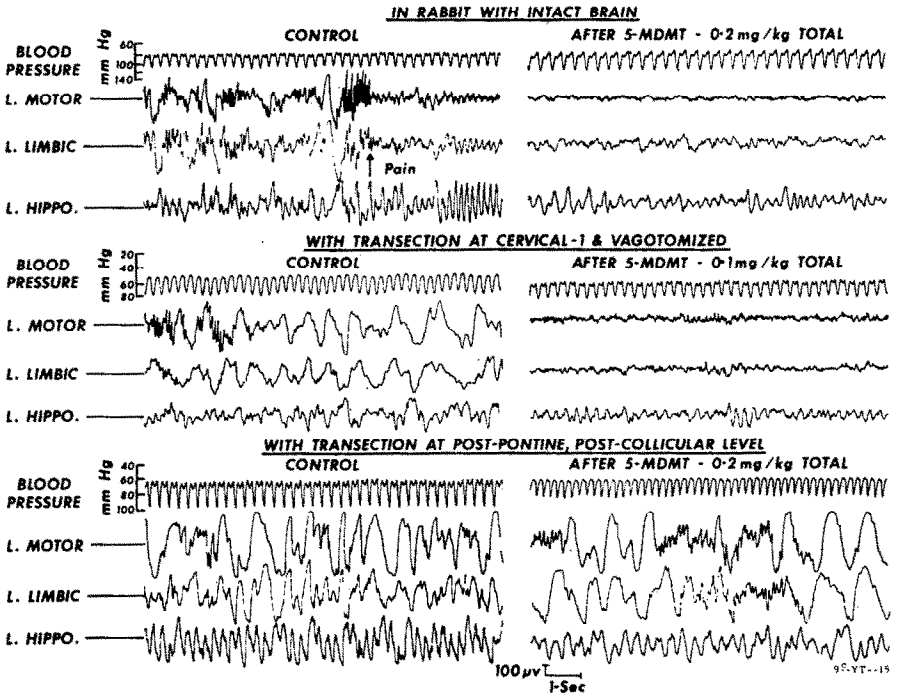

FIG. 4. EEG effects of 5-methoxy-*N*-dimethyltryptamine (5-MDMT) in rabbits. Control on left side. On right side note the EEG arousal pattern with depressed amplitude of the theta hippocampus rhythm after 5-MDMT. These EEG arousal patterns are observed in rabbits with an intact brain as well as with vagotomized rabbits transected at C1. On the contrary, an EEG arousal pattern was not produced after transection just below the midbrain.

total dosage of 0·1 mg/kg (repeated injections of 0·025 mg/kg), evoked modified EEG arousal patterns for 10 min in one animal. In four other rabbits given repeated doses of 0·05–0·2 mg/kg, modified EEG arousal patterns also were maintained for periods ranging from 2 to 30 min; 5-MDMT also was injected in still another four rabbits at 0·1 mg/kg. Each injection produced brief typical EEG arousal patterns which were sustained for 0·5–2 min and were accompanied by somewhat decreased amplitude theta waves in the hippocampus during the next 3–8 min. After EEG arousal patterns were initiated, either typical or modified EEG arousal patterns persisted for approximately 10 min. In each of four rabbits, 0·2–0·3 mg/kg is total doses (repeated injections with 0·1 mg/kg) evoked either typical or modified

EEG arousal patterns for 10–45 min. The blood pressure levels were immediately elevated by 10–20 mm Hg for about 2 min after the injections and then returned to original levels. A single injection at a higher dose of 0·5 mg/kg in one animal produced EEG changes consisting of moderate amplitude slow waves in all leads, associated with hypotension and cardiac arrhythmias. In three denervated preparations with total dosages of 0·1–0·2 mg/kg (repeated injections of either 0·05 or 0·1 mg/kg) and therefore lower dosages than those in the preparations with intact carotid functions, strong EEG arousal patterns were observed in all animals studied.

Moreover, in each of four preparations transected at C1 with additional bilateral vagotomy, remarkable EEG arousal patterns were obtained after total dosages of from 0·1 to 0·25 mg/kg. But even higher doses (0·4–0·5 mg/kg) of this agent after postpontine, postcollicular transections failed to induce any EEG changes in each of five preparations.

In view of the fact that the oxalates of DMT and 5-MDMT were administered, it is well to add that when oxalic acid was injected i.v. in doses of 2 mg/kg in three rabbits, no changes occurred except for a brief initial EEG arousal similar to that observed in the control animals with intact brain on brief peripheral stimulation.

DISCUSSION

Our results show that 5-methoxytryptamine (5-MT), *N*-dimethyltryptamine (DMT) and 5-methoxy-*N*-dimethyltryptamine (5-MDMT) induced EEG arousal patterns in rabbits with intact brain. The minimal effective doses of 5-MT, DMT, and 5-MDMT as the bases were 0·2–0·5 mg/kg, 1·3–3·2 mg/kg, and 0·14–0·21 mg/kg respectively. The short-lasting actions of 5-MT and the failure to obtain cumulative effects after repeated injections at 7-min intervals indicate a rapid inactivation of this agent. The EEG arousal induced by DMT consisted of initial short-lasting and delayed long-lasting patterns in most of the animals with intact brain. The initial EEG arousal patterns were associated with pressor responses which appeared immediately after injection. But impairment of this initial EEG arousal pattern was observed in association with the reduced pressor response when DMT was repeated at 7-min intervals. The initial EEG arousal patterns may be due to changes in cerebral blood flow resulting from rises in blood pressure or to reflex effect from peripheral receptors. Bradycardia and other reflex actions via carotid sinus and vagal nerves following the pressor responses seemed partially responsible for the resting pattern and for the delay of the control EEG arousal pattern, because the denervation and vagotomy diminished the duration of the resting patterns intervening between the initial and the delayed EEG arousal patterns. But even in denervated preparations, EEG arousal patterns assumed more typical forms 5–8 min after injection. This observation suggests that DMT does not penetrate into the brain readily or may act via some intermediate metabolite. Szara and Hearst[11] and Szara *et al.*[12] postulated that 6-hydroxylation of tryptamine derivatives may be a possible pathway for the production of psychoactive metabolites. However, Rosenberg *et al.*[13] could not find psychotomimetic activity for 6-hydroxy-DMT in human subjects. In contrast to the results with DMT, each injection of 5-MDMT evoked EEG arousal patterns which became more marked and most sustained as the dosage was repeated. The EEG arousal that occurred for brief periods shortly after each injection may be associated with rises in blood pressure like those after DMT injections. These EEG arousal

patterns associated with hypertension were followed by another kind of EEG arousal pattern when premedication levels of blood pressure were restored. EEG arousal patterns induced by 5-MDMT also seem to be partially masked by loss of reflex action via sinus and vagal nerves in denervated preparations. In fact, the stimulation of baroreceptors is known to induce inhibition of the reticular formation.[14] Tryptamine did not induce EEG changes except in low-nontoxic doses when short-lasting EEG arousal patterns were only rarely observed and then simultaneously with the pressor response. From the dosages used to evoke EEG arousal patterns it would seem that 5-MDMT is more potent than DMT. Similarly, 5-MT is more potent than tryptamine. Thus mono-*O*-methylation of an indoleamine in the 5-position increases potency for evoking EEG arousal.

Studies in organic chemistry reveal that *O*-methylation and *N*-methylation increase lipid solubility. Taking into consideration the permeability of the blood–brain barrier, we suggest that the greater relative potencies of 5-MDMT and 5-MT may be ascribed in part to their better penetration into the brain. Axelrod[15] found that serotonin and tryptamine are enzymatically *N*-methylated to form the psychotomimetic metabolites bufotenin and DMT. Furthermore, dihydroxyindoles have been found to be *O*-methylated in the 5- and 6-positions[16] by catechol-*O*-methyltransferase. In addition, our present results make it possible to speculate on the formation of *O*-methylated indoleamines with potent central actions.

Transections of the brain revealed that 5-MT induced EEG arousal patterns in a midbrain site, whereas the main site of EEG arousal patterns induced by DMT and 5-MDMT is in the medullary region. The finding that 5-MT induced EEG arousal patterns at the mesencephalic level suggests that the introduction of one methoxy group at the 5-position of indoleamines is not adequate to shift the site of EEG arousal patterns to a medullary site. It is possible, however, that the presence of two or more methoxy groups in indoleamines might shift the site of EEG arousal patterns to the medulla in analogy to our previous observations with catecholamines.[1, 9] In that investigation, 4-methoxyphenylethylamine provoked EEG arousal in the midbrain, in contrast to mescaline and 3,4-dimethoxyphenylethylamine. The latter two compounds, with three and two methoxy groups respectively, exhibited a medullary site for the arousal reaction. A similar relationship was observed between *N*-monoethyl and *N*-diethyl derivatives of lysergic acid amide.[2]

Finally, we would like to point out that the psychotomimetic activity of DMT has been well established in man.[17] 5-MDMT has been found to be the principal active component of Epena, which is used by South American Indian tribes as psychotomimetic snuff.[8] The present results provide additional evidence for our hypothesis that most psychotomimetic catecholamines and indoleamines induce EEG arousal patterns at the medullary level.[1–4, 9] It is conceivable that DMT and 5-MDMT evoke EEG arousal patterns either by stimulating a site in the medullary reticular formation or by inhibiting a bulbar synchronizing mechanism.[18–21]

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