CHAPTER 13

Behavioral, EEG and Biochemical Variations After the Administration of Alpha-Methyltryptamine (IT 403)

By W. G. VAN METER, A.B., E. COSTA, M.D., G. F. AYALA, M.D., AND H. E. HIMWICH, M.D.

BEHAVIORAL ABNORMALITIES, and biochemical changes have been observed after the administration of the experimental drugs IT 403 (alpha-methyltryptamine) and IT 290, a weaker racemic form of IT 403.* Stimulation of the central nervous system caused by IT 403 has been noted in several species of animals.[1] Moreover, Grieg et al.[2] have reported that alpha-methyltryptamine inhibits monoamine oxidase as well as 5-hydroxytryptophan decarboxylase in vivo and in vitro. The structural relationship between IT 403 and scrotonin (5HT) are shown in FIGURE 1. However, the biological and physiochemical characteristics of alpha-methyltryptamine reveal that this drug, unlike its chemical congener 5HT, readily passes the blood-brain barrier. This property and the prompt inhibitory effect of IT 403 on MAO (monoamine oxidase) with resulting accumulation of biogenic amines indicates that this drug lends itself for investigating the EEG activation that is induced in brain sites by MAOI (monoamine oxidase inhibitors).

The biochemical and electrophysiologic effects of amphetamine, an EEG alerting drug[3] without monoamine oxidase inhibition activity and Harmine,[4] an EEG activating drug[5] with well known, short-acting, reversible MAOI properties[6] were compared with the experimental results of IT 403 as an aid in the evaluation of a site of action in the brain for the latter compound. In addition, these drugs were injected through the carotid or vertebral arteries of rabbits prepared with the basilar artery ligated at the midpontine level. Injections given in this manner distributed the drugs in the brain either anterior or posterior to the level of the tied basilar and thereby per-

We are grateful to Doctor Ottillie Inman for preparation of the sections of the brain demonstrating distribution of the Monastral blue dye.

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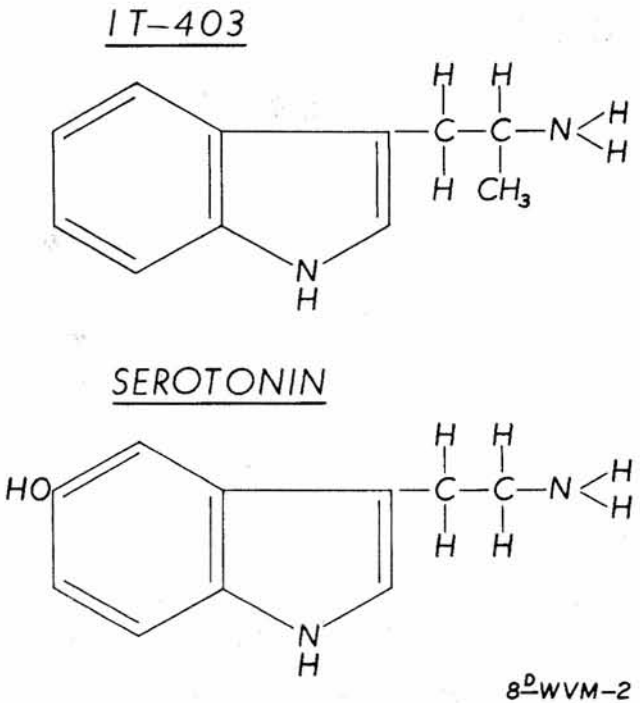

FIG. 1.—Structural relationship between IT 403 (alpha-methyltryptamine) and serotonin (5-hydroxytryptamine). Note the similarity in structure of IT 403 and serotonin with the exception of the OH group in the 5 position on the benzene ring and the methyl group in the alpha position on the side chain.

mitted a more direct evaluation of the central site and mechanism of action of alpha-methyltryptamine.[7]

METHODS

The adult New Zealand albino rabbits used in this study were tracheotomized under ether and local pontocaine anesthesia, curarized and artificially respired.[8] Ether anesthesia was discontinued after all surgical procedures were completed and a sufficient interval of time was allowed to elapse for the rabbit to eliminate the ether.

Electrophysiologic responses to alpha-methyltryptamine were observed in one group of intact rabbits receiving the drug intravenously either through the marginal ear vein or via a polyethylene cannula inserted into the femoral vein. In another group of rabbits the basilar artery was exposed and ligated at the midpontine level thereby dividing the arterial circulation of the brain into two parts and making it possible to limit direct arterial injections into

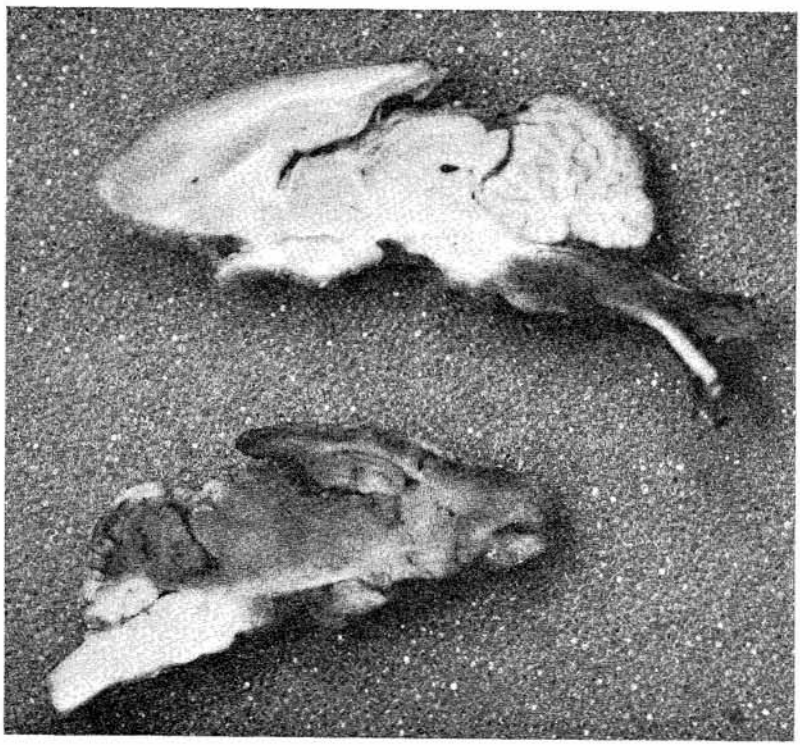

FIG. 2.—Distribution of monastral blue dye injected arterially through the vertebral artery (upper saggital section) or the carotid artery (lower saggital section). Note that the vertebral injection in the upper brain slice is limited rostrally to the region of the bulbar reticular formation while the carotid injection includes the midbrain reticular formation as its caudal border.

the brain either cephalad or caudad to the level of ligature. Intracarotid injections infused the forebrain anterior to the basilar tie and intravertebral injections posterior to the tie as shown in FIGURE 2. In the last group of animals, the brain stem was transected with a silver wire at the level of the basilar ligature. Alpha-methyltryptamine was administered to these animals intravenously, intravertebrally, or through the carotid artery as diagramed in FIGURE 3 and described in another paper.[7] Arterial injections were made through a polyethylene bypass in the common carotid artery or the cannula in the subclavian artery of rabbits with the basilar artery ligated at the midpontine level. The amount of drug injected by the intravenous route is expressed in reference to body weight, i.e. 3 mg./Kg., but the arterial administration is given as total amount of drug, i.e., 3.0 mg. total and not referred to body weight. The EEG tracings from cortical and subcortical

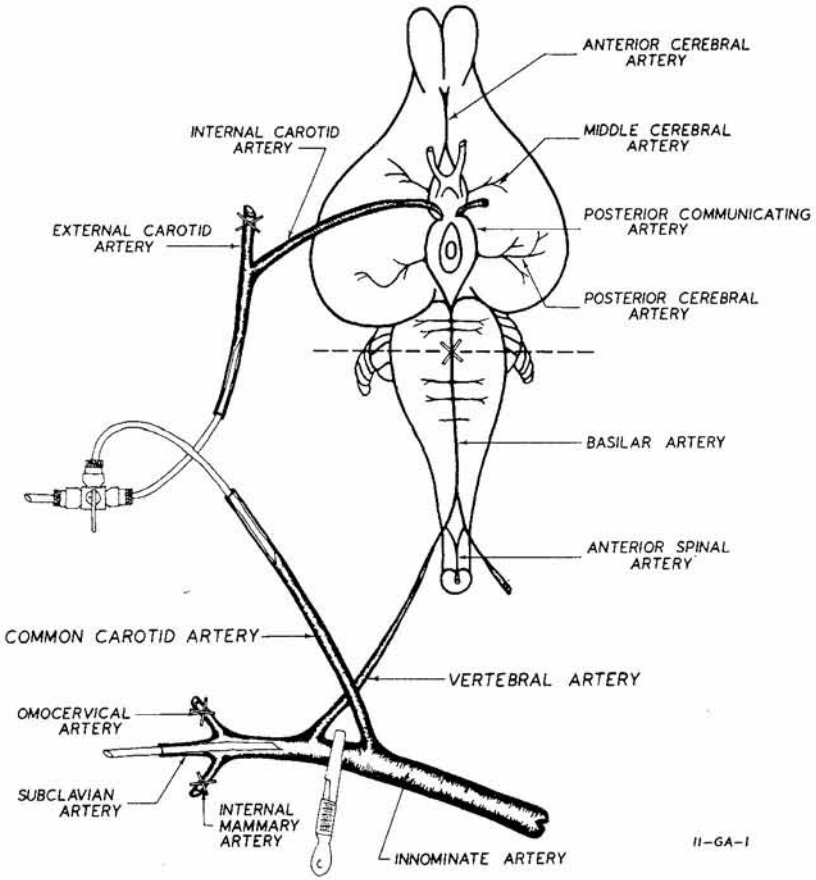

FIG. 3.—Diagram of the method of intracarotid or intravertebral arterial injections in rabbits with the basilar artery ligated at the midpontine level (– –x– –). Intravertebral administration of drugs is made through a polyethylene cannula inserted into the subclavian artery. A bulldog clamp is placed on the subclavian artery between the points of origin of the vertebral artery and the common carotid artery only during the time of injection. These injections are limited to the vertebral artery including the area of the brain caudal to the position of the basilar ligature. Intracarotid arterial injections are given through the polyethylene bypass and they irrigate the portion of the brain anterior to the level of basilar ligature. The stopcock is turned to restore normal circulation, immediately after the injection of the drug.

structures, as identified in FIGURE 4, are taken with a Grass Model III D electroencephalograph.

When the brain 5HT of these rabbits was to be determined by bioassay, the brains were perfused with physiologic saline, extirpated and the cerebral cortex, hippocampus, caudate nucleus, cerebellum and brain stem were dissected and frozen on dry ice.[9,10]

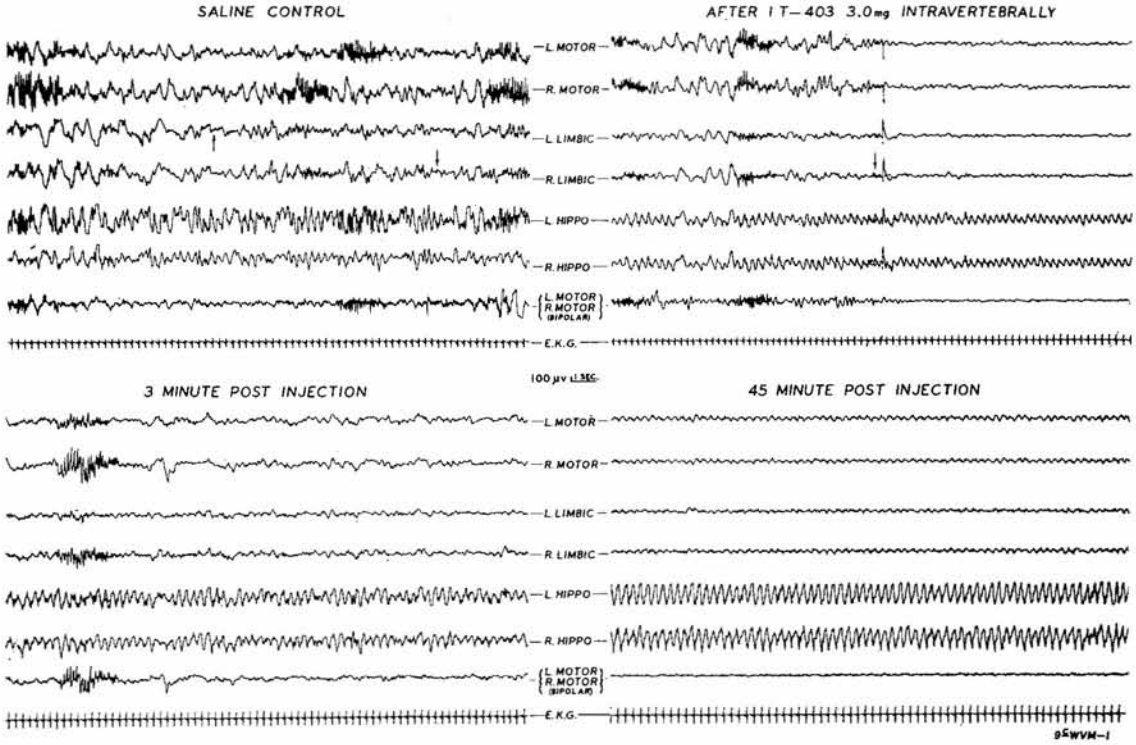

FIG. 4.—EEG arousal evoked by intravertebral arterial administration of IT 403 (alpha-methyltryptamine) in rabbits with the basilar artery tied at the midpontine level. Upper left group of tracings—Saline control. The effect of 1.0 mg. of saline injected intra vertebrally (*arrow*) does not change the spontaneous recording EEG. After IT 403 3.0 mg. intravertebrally (upper right group of tracings). Note the EEG arousal evoked by the injection (*arrow*). Three minutes past injection (lower left group of tracings). The spontaneous EEG activity returns to a sleep tracing with 14 cps spindles and higher amplitude slow waves within 2 to 3 minutes after the intravertebral injection of 3.0 mg. of IT 403. Forty-five minutes past injection (lower right group of tracings). The secondary EEG arousal occurs with significant increases in brain biogenic amines and persists at least one hour after its onset. Leads are as follows: L and R motor—left and right motor cortex, respectively. L and R limbic—left and right limbic cortices respectively. L hippo and R hippo—left and right dorsal hippocampus respectively. EKG—electrocardiogram.

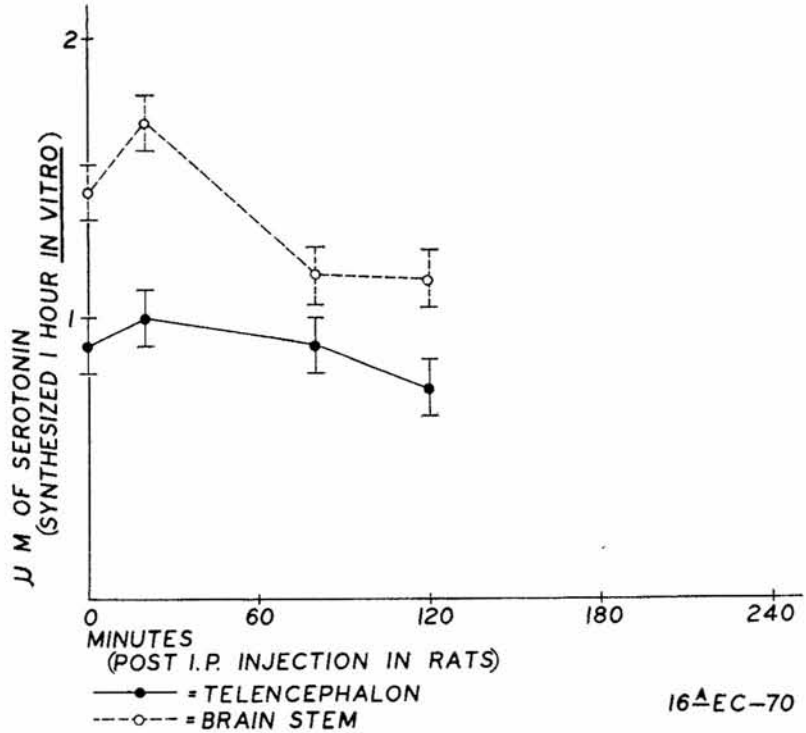

FIG. 5.—In vivo inhibition of brain 5 HTP decarboxylase in rats following the administration of IT 403 20.0 mg./Kg. i. p. Note the minimal inhibition of 5 HTP decarboxylase in brain stem (open circles) that returns to normal with one hour and the lack of inhibition that occurs in the telencephalon (closed circles).

Rats also were used in this investigation and bioassay methods measured the in vivo inhibition of brain 5HTP decarboxylase.[11] The extent of MAOI activity measured by bioassay methods was gauged by increases in brain 5HT content of these animals. The EEG and biochemical effects of IT 290 and IT 403 were compared with those of amphetamine as well as the two MAOI, Catron (beta-phenylisopropyl hydrazine) and Harmine. However, IT 403 is more active than the racemic form, IT 290 and consequently has been more intensively investigated (FIGURE 5).

RESULTS

EEG

The EEG response to the intravenous administration of 3 mg./Kg. of alpha-methyltryptamine to intact and *cerveau isolé* rabbits was an immediate arousal pattern of low amplitude and fast frequency. This arousal persisted

up to 2 hours. Intracarotid injections of 3 mg. of IT 403 in intact or *cerveau isolé* rabbits evoked an immediate EEG arousal similar in all respects with the EEG responses seen after intravenous injection. In contrast, intravertebral administration of 3 mg. of IT 403 produced different results from those seen after either the intracarotid or intravenous injections. An immediate, transient, EEG arousal occurred with the injection. This lasted for about two minutes and was followed by a shift in the brain tracings toward sleep with increased amplitude and the reappearance of 12 to 14 cps spindles. This sleep pattern endured for about 40 minutes and then reverted to a complete arousal which persisted at least one hour (FIG. 4). While still aroused, the animal was sacrificed and brain samples were removed.

Harmine 1.0 mg. given intravertebrally to rabbits elicited an EEG arousal beginning about 10 minutes after the injection. This EEG pattern continued until the animal was sacrificed for analysis of brain 5HT content ten minutes later at a time when the EEG was still activated. In contrast, Harmine given into the carotid catheter, even in doses of 2.0 mg. failed to evoke an EEG alert pattern over a period of one hour.

Conversely, amphetamine injected either intravenously or intracarotidly showed an EEG activation but failed to elicit an EEG arousal via the verte-

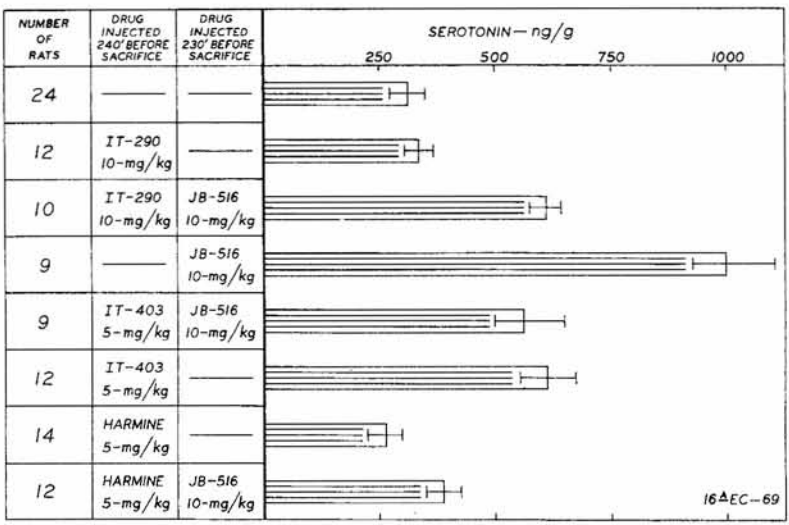

FIG. 6.—Brain serotonin concentrations of rats used as measurement of in vivo inhibition of rat brain MAO caused by IT 290, IT 403, Harmine or JB 516 (Catron) given intraperitoneally. *Horizontal rows:* The control value of brain serotonin (row 1); compared with inhibition of MAO evoked by IT 290 (row 2); by JB 516 (row 4); IT 403 (row 6); and Harmine (row 7). Note the JB 516 serotonin rise in row 4 and the lessening of this expected increase caused by pretreatment with IT 290 (row 3), IT 403 (row 5) and Harmine (row 8).

bral artery. Occasionally, after the intravertebral injection the EEG pattern revealed transient alerting of 20 to 30 seconds duration followed immediately by a return to the EEG sleep pattern which persisted until the drug had sufficient time to be distributed systemically and a generalized EEG arousal occurred.

Biochemical Results

In agreement with the report of Grieg et al.[2] 20 mg./Kg. i.p., of IT 403 elicited a minimal inhibition of 5HTP decarboxylase of rat brain (FIG. 5). FIGURE 6 illustrates some of the characteristics of brain MAO inhibition caused by methyltryptamine in rats. Like Harmine[6] IT 403 prevented the increase of brain 5HT caused by Catron given 10 minutes after IT 403.

Three mg. of IT 403 administered intravertebrally to rabbits induced a secondary EEG arousal after a latency period of about 30 minutes. At the moment of this arousal the 5HT content of the brain stem might be increased[15] but no strict correlation could be made between brain stem 5HT increase and arousal (FIG. 7). No changes of the 5HT in brain stem occurred with the EEG arousal (1 hour) that appeared immediately after the *intracarotid* injection of 3 mg. of IT 403 (FIG. 7). On the other hand, Harmine, 1.0 mg, given *intravertebrally* to rabbits caused both an increase of brain stem serotonin and an EEG arousal within 20 minutes. In contrast, *intracarotid* injections of

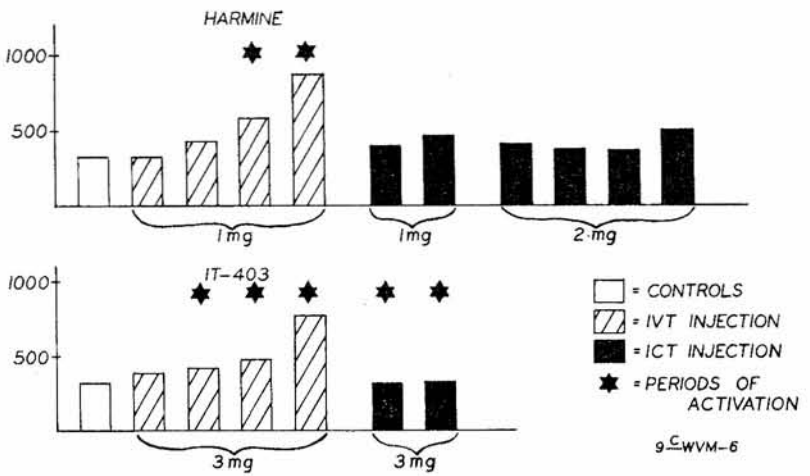

FIG. 7.—Comparison of EEG effects and biochemical alterations after Harmine and IT 403. Upper graph: note that an EEG arousal (*stars*) and significant increases in brain stem serotonin (*lined bars*) occur with intravertebral arterial injections of Harmine 1.0 mg., but not after intracarotid injections (1.0 mg. or 2.0 mg. solid bars). Lower graph: 3.0 mg. of IT 403 given intravertebrally (*lined bars*) or intracarotidly (*solid bars*) evokes an EEG arousal, (*stars*) but significant accumulation of brain stem serotonin occurs only with intravertebral injections (ng/g of tissue).

1 or 2 mg. induced neither an EEG arousal nor an increase in brain stem serotinin (FIG. 7). Amphetamine does not alter the brain stem 5HT content.[4]

DISCUSSION

Clinical investigations have shown that MAOI reveal specific patterns of central nervous system stimulation. The studies of Alexander and Berkley,[12] Ayd,[13] and Bailey et al.[14] have revealed the value of monoamine oxidase inhibitors in the treatment of depressions.

Behavioral observations reported by the Sandoz Laboratories[1] revealed that IT 403 given subcutaneously to cats in amounts of 1.0 to 3.0 mg./Kg. elicited a long-lasting fear reaction during which the animal retired to a corner of its cage, carefully observed its environment and failed to react when a mouse was placed in the cage. In addition, dogs became markedly disturbed following the administration of IT 403 and exhibited suppression of appetite. Mice also revealed behavioral abnormalities after being given this drug, the most prominent of which was increased motor excitation. These abnormal behavioral effects observed in experimental animals given IT 403 may be attributed, in part, to an increased excitatory response in the midbrain reticular formation.

IT 403 evoked a minimal inhibition of 5HTP decarboxylase that was insignificant and probably does not account for the pharmacological activity of this drug. The most prominent pharmacological action observed after the administration of IT 403 was the inhibition of monoamine oxidase which resulted in a quantitatively significant increase in brain serotonin. This increased concentration of serotonin in the brains of rats and rabbits, after the administration of IT 403, receded after a period of about four hours. Moreover, if IT 403 was compared with beta-phenylisopropyl hydrazine HCl (Catron, Lakeside Laboratories) a more potent, longer acting monoamine oxidase inhibitor, a competitive inhibition was demonstrated (FIG. 6). The expected increase of brain serotonin evoked by the longer acting monoamine oxidase inhibitor was prevented if IT 403 was given 10 minutes prior to the administration of Catron. Though both drugs inhibit MAO it may be that the diminution of the Catron-evoked serotonin increase is a result of the initial action of IT 403 on the receptor site of the enzyme MAO thus blocking it from further attack by the longer acting Catron. These results are in agreement with the observations of Pletscher[6] who noted that the expected increase of 5HT evoked by the long-acting iproniazid (beta-isonicotinic acid isopropyl hydrazide) was diminished if the animals were pretreated with Harmine and suggested the existence of a competitive inhibition between long-acting and short-acting MAOI.

Therefore, the action of IT 403 on brain biogenic amines shows that it is reversible, short-acting monoamine oxidase inhibitor that resembles in

many respects the reversible, monoamine oxidase inhibition properties of Harmine.

The effect of alpha-methyltryptamine on the MDAS (mesodiencephalic activating system) was suggested by the immediate EEG arousal evoked after the *intravenous* administration of the drug. This arousal was immediate and persisted up to two hours in both intact and *cerveau isolé* preparations. More direct evidence of an influence on the MDAS was revealed by intracarotid injections of IT 403 in intact animals and in rabbits with the brain stem transected at the level of the basilar ligature. The drug continued to elicit an EEG arousal analogous to that seen after intravenous injections. In contrast, intravertebral injections of the drug evoked an immediate, transient EEG arousal, but this pattern persisted for only about two minutes after which time the EEG tracings showed the characteristics of low amplitude sleep waves with intermittent 14 cps spindle activity. This period of EEG sleep continued for approximately 40 minutes. The brain waves then resumed an EEG arousal pattern that persisted for at least one hour. Therefore, though the intravertebral administration of IT 403 affects the MDAS by evoking an EEG arousal response, this response includes an initial, brief alert pattern that occurs on injection and persists for two minutes or less as well as a secondary longer lasting arousal that occurs approximately 40 minutes later and persists for at least one hour. The mechanism of the brief (two minutes or less) initial EEG arousal is not clear.

If the biochemical correlates are taken into consideration it is noted that the initial phase of the EEG arousal induced by alpha-methyltryptamine occurs at a time when brain biogenic amines are within normal values. However, the latter phase of the arousal pattern, as revealed by the intravertebral injections of alpha-methyltryptamine, is associated with a large increase in brain stem 5HT.

The mechanisms by which increases in brain 5HT cause EEG activation[15] are not understood. It is worth mentioning that Batini et al.,[16] have described, in the lower brain stem, an inhibitory center, the depression of which can produce increased activation and this suggests a working hypothesis that would explain the action of MAO inhibitors. Moreover, our data with Harmine revealed that the intravenous or intravertebral administration of this drug elicited an EEG arousal with a significant increase in brain 5HT, while intracarotid injections neither altered significantly the EEG nor concentrations of the brain stem biogenic amines. These observations give evidence that MAOI effects can take place in the pontine and medullary regions of the reticular formation.

Furthermore, the caudal region of the reticular formation does not appear to be a locus of action for the non-MAOI EEG alerting drug amphetamine. Amphetamine does not alter the brain 5HT and shows an EEG arousal when

given intracarotidly or intravenously to intact or *cerveau isolé* rabbits prepared with the basilar artery ligated at the midpontine level. Intravertebral administration of this drug does not produce an EEG arousal nor alter the brain biogenic amine 5HT. The fact that amphetamine was more effective when given *intracarotidly* is in agreement with the findings of other authors[3] who favor a more anterior site of action in the reticular formation for amphetamine. In fact, Hiebel et al.[17] have demonstrated that amphetamine continues to elicit an EEG arousal unless the cephalad portion of the brain stem is transected in a plane that passes through the posterior third of the thalamus to the base of the brain in front of the mammillary bodies.

The results of our EEG studies show that the midbrain reticular formation is involved in the EEG arousal response following administration of IT 403. The mechanism has at least two aspects, involving an initial phase of EEG arousal without 5HT rise and finally a more prolonged EEG alerting occurring with large increases in brain stem serotonin. In addition, our experimental results indicate that the EEG activation induced by MAOI differs from that caused by amphetamine with regard to the site of action. The former may act in regions of the reticular formation which are more caudad than those predominantly affected by amphetamine. It is concluded that alpha-methyltryptamine causes EEG activation by a bipartite action involving both amphetamine-like and Harmine-like properties of this drug.

Summary and Conclusions

A neurophysiologic analysis has been made of alpha-methyltryptamine (IT 403 and IT 290) on rabbit with intact brain and *cerveau isolé* preparations. Previous work demonstrating that these drugs are short-acting, reversible monoamine oxidase inhibitors was confirmed and extended by studies which disclosed that alpha-methyltryptamine injected before Catron diminished the monoamine oxidase inhibitory effects of the latter.

The EEG observations revealed activation as a result of intravenous injections. However, if the drugs were given arterially to rabbits with the basilar artery tied at the midpontine level so that intravertebral injections were limited to the brain posterior to the ligature and intracarotid injections restricted to the region of the brain rostral to the midpontine tie, differences in the EEG alerting were observed. Intracarotid injections of IT 403 evoked an immediate alert pattern in intact as well as *cerveau isolé* preparation, while the intravertebral route of drug administration elicited a long-lasting arousal that began approximately 30 minutes after the injection. The EEG arousal following the intracarotid route of injection was not accompanied by increases in the brain 5 HT while intravertebral injections were accompanied by changes in the brain serotonin.

It is concluded that the activation caused by IT 403 and IT 290 in the

intact animal is bipartite in character and includes two phases: one starting immediately after injection and resembles the type of activation produced by amphetamine, involving the midbrain reticular formation; and a delayed phase associated with the effects of the raised level of serotonin on the bulbar reticular formation. The total effect of IT 403 and IT 290 on the EEG response of the intact animal, therefore, involves a combination of these two actions.

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