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Behavioral Alterations Induced by Formaldehyde-Derived Tetrahydroisoquinolines

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MAKOWSKI, E. C. AND L. A. ORDOÑEZ. *Behavioral alterations induced by formaldehyde-derived tetrahydroisoquinolines*. PHARMAC. BIOCHEM. BEHAV. **14**(5) 639–643, 1981.—Rats administered intracerebroventricular (IVT) injections of 3-Carboxy-1,2,3,4-tetrahydroisoquinoline (I) or 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline (II), the condensation products of formaldehyde with phenylalanine and dopamine, respectively, were evaluated in the open field and shuttle box situation. In the open field, 45 min after the acute administration of I, several repetitive purposeless responses and other manifestations such as teeth chattering, wet dog shakes and stiffness of the tail were observed; however, no quantitative effects were detected in the behavioral parameters recorded. Further studies were performed utilizing the shuttle box situation with drugs I and II. They produced an increase in the number of escapes and latency of shocks when measured 45 min after IVT administration (10 mg/ml and 1 mg/ml, respectively; 20 μl). Our data suggest that the studied compounds induce qualitative alterations as well as changes in shuttle avoidance behavior in the rat when measured 45 min after administered. The effects were found to be dose and time dependent, being reversible when measured 24 and 48 hours after administration of the drugs. The present results open the possibility that the *in vivo* formation of tetrahydroisoquinolines could be involved in some mental pathologies, such as schizophrenia, as has been previously suggested.

Intraventricular injections Tetrahydroisoquinoline alkaloids 3-Carboxy-1,2,3,4-tetrahydroisoquinoline 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline Open field Shuttle avoidance behavior

IN the last decade special attention has been given to Tetrahydroisoquinolines (TIQs), the condensation products of biogenic amines and aldehydes, as there is ample evidence regarding the behavioral alterations induced by these alkaloids in experimental animals. Most of the reports in this field suggest an involvement of TIQ derivatives in the biochemical mechanisms underlying alcohol addiction [1, 3, 8]. In this regard it has been reported that the chronic infusion of acetaldehyde-derived TIQs into the lateral ventricle of the rat induces an increase in voluntary alcohol intake [7, 8, 9, 10]. In order to support this point, the same group also reports behavioral alterations which resemble stereotypical responses such as tremor, rigidity, head shaking, teeth chattering, stiffness of the tail, wet dog shakes and hyperactivity. With prolonged IVT infusions of TIQs audiogenic seizures and running episodes have been observed [7, 9, 10]. Melchior and Myers [7] reported that the acute administration of 40 μg of tetrahydropapaveroline (THP; 20 μl) produce no significant changes in alcohol intake, indicating that an element of chronicity is required in the induction of drinking by the amine condensation product acting within brain. On the other hand, a dose related decrease in the rate of fixed ratio responding after chronic administration of salsolinol (15–120 μg), another acetaldehyde-derived alkaloid, has also been reported [5]. Furthermore, Costall *et al.* [2] report that the bilateral injection of up to 100 μg acetaldehyde-derived TIQs into the nucleus accumbens of the rat tends to cause hyperactivity, but when formaldehyde-derived TIQs were injected after a nialamide pretreatment virtually no changes in locomotor activity were observed.

Our laboratory has put forward a hypothesis [12] that suggests that the increased generation within brain of methylenetetrahydrofolic acid in conditions which do not allow its utilization by normal metabolic pathways would produce an increased generation of formaldehyde in the tissue, which could lead through reaction with amine groups, to TIQ formation, which in turn could be responsible, at least in part, for some of the psychotic manifestations seen in schizophrenia. By this mechanism formaldehyde could participate in the generation of some TIQs with behavioral effects on biological organisms. Therefore, we have measured the behavioral alterations induced in the rats by 3-Carboxy-1,2,3,4-TIQ (I), the condensation product of formaldehyde with phenylalanine, and 6,7-Dihydroxy-1,2,3,4-TIQ (II),

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produced by the reaction of formaldehyde with dopamine. The results of these studies are presented in the following sections.

METHOD

Animals

Naive male albino Sprague-Dawley rats, born in the animal colony of the Instituto de Medicina Experimental from the Universidad Central de Venezuela, weighing 250 g approximately, were provided Ratarina® diet and water ad lib.

Drugs

Both TIQ derivatives (hydrochloride salts) used in the present experiments were synthetized and purified in our laboratory. I, the condensation product of phenylalanine and formaldehyde, was synthetized according to Julian *et al.* [6]; II was produced by the reaction of dopamine with formaldehyde following the procedure of Heikkila *et al.* [4]. The purity of both derivatives was determined by melting point determinations and thin layer chromatographic analysis.

Both compounds were dissolved in saline, requiring the addition of HCl in the case of II. The resultant pH of both solutions was made 1.78; therefore, normal saline, adjusted to pH 1.78 with HCl, was used as control. For each experiment solutions were freshly prepared.

Phenylalanine and dopamine were purchased from Sigma Chemical Company.

Apparatus

Open field situation, which consisted in a plastified wood box of 122 cm length, 122 cm wide and 32 cm depth. The bottom was marked in 36 equal squared areas measuring 20.3 cm each. The field was illuminated with a 15 W light located 1.6 m above the floor. The visibility of all the surface was possible since the box was uncovered.

The behavioral parameters recorded were *latency*: time in seconds animals would delay in leaving the central square; *activity*: measured as the number of marked floor segments that each animal entered with his two front paws at least; *rearing*: number of times each rat had both front paws off the floor at the same time except when grooming; *defecation*: total number of fecal boluses dropped by each rat.

Shuttle box, purchased from Lehigh-Valley Electronics (BRS/LVE RSC-004 "Rat Toogle Floor Shuttle Cage"). It consists of a two compartment cage for avoidance training and a center partition with a 6.3 cm round opening separation between both chambers; a 1.4 mA foot shock was delivered through the floor grid. The discriminative stimulus was a 3 W light placed in the extreme wall of each compartment. The behavioral parameters recorded were *number of escapes*: the number of times an animal crossed from one compartment to the other in the presence of the aversive stimulus (shock, AvS); and, *latency of shocks*: the time in seconds that an animal delayed in crossing from one compartment to the other in the presence of the AvS.

Statistics

Student's *t*-test was used to compare experimental and control groups. On the other hand, individual baselines were recorded, serving as individual control values.

Procedures

All animals were equally trained, then assigned randomly to experimental (drugs) or control (saline) groups; each animal received a single injection of either drug or saline in order to study drug effects. Intraventricular procedure and post-IVT treatment was again similar for both, experimental and control groups. Previous experiments were run in which non-injected controls (with or without ether administration) were compared to saline groups, observing that all animals were comparable as controls when behavioral recordings were performed 45 min after ether administration.

Solutions were administered to animals following the method described by Noble *et al.* [11]. Hamilton microsyringes bearing 27-gauge needles with stops at 2.5 mm from the needle tip were utilized for IVT administration. The animals were anesthetized with ether, gently restrained, the scalp was incised, and 20 μl of isotonic saline or drug solution were administered into the right lateral ventricle at a rate of 2 μl/sec. The success frequency of IVT hits was determined by methylene blue dye injections in a separate group of animals.

In the open field situation, all animals (n=42) received a 10 min habituation session after which they received a 5 min trial per day on 10 successive days. On day 11, drug I (10 mg/ml; 20 μl) was administered and behavioral parameters registered 45 min later, after all anesthetic effects had disappeared. It was previously determined that ether effects disappeared within 30 min after exposure of the animals to the anesthetic. As additional controls, groups of non-injected animals (n=12) were tested in the field for 20 days and no changes in their baselines were observed.

In the shuttle box situation, all the rats were trained in a 2-way active shuttle avoidance for 20 trials every 48 hr on at least 10 occasions. On a given acquisition trial, the onset of a 3 W light used as the discriminative stimulus (DS) was followed 20 sec later by a 1.4 mA foot shock (AvS) unless a shuttle response was emitted, which precluded the shock onset and terminated the DS (avoidance). Responses made in the 30 sec period after the AvS onset coterminated both the light and the shock (escape). Trials were separated by a 30 sec fixed interval, and intertrial responses did not affect avoidance contingency. On the day of the experiment, a single IVT injection of drug I (10 mg/ml; 20 μl) or drug II (10 or 1 mg/ml; 20 μl) was administered and behavioral parameters registered 45 min, 90 min, and 2 hr later in different groups of animals.

All observations with drugs and controls were performed under blind conditions.

RESULTS

Open Field

Forty-five minutes after drug I (10 mg/ml; 20 μl) or saline (20 μl) administration the observers could distinguish groups of animals that presented repetitive purposeless responses and other manifestations such as teeth chattering, wet dog shakes and stiffness of the tail, which, upon later examination, proved to be the experimental subjects receiving the drug (qualitative observations).

When the recorded parameters: latency, activity, rearing and defecation were quantitatively analyzed no differences between experimental and control animals were detected, probably due to intra- and inter-individual variability, as well as non-consistent effects of drug or saline treatments on

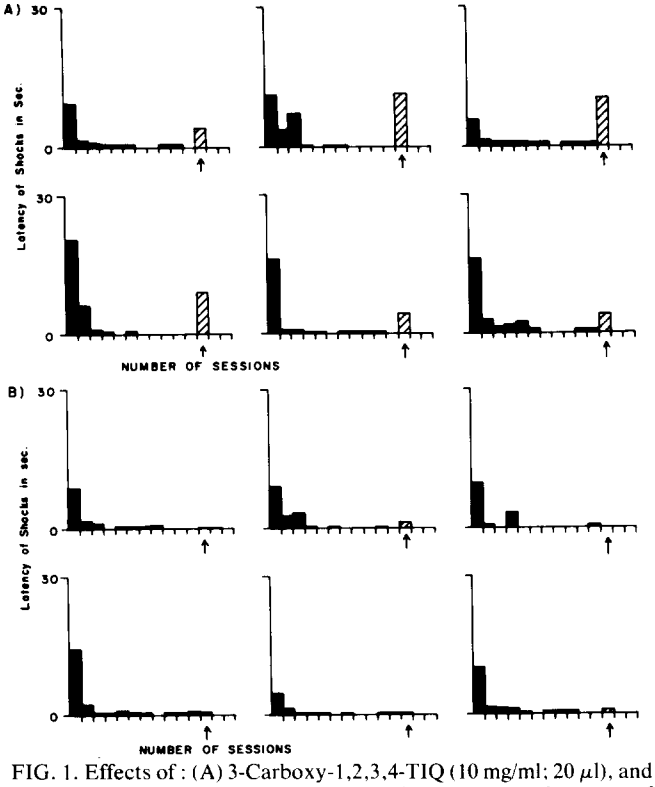

FIG. 1. Effects of: (A) 3-Carboxy-1,2,3,4-TIQ (10 mg/ml; 20 μl), and (B) NaCl (0.9%; pH 1.78; 20 μl) on the parameter Latency of Shocks, measured in the Shuttle Box situation, 45 min after administration of the drug. The arrows indicate the day session on which injection was administered to each animal. Absence of bars indicates 100% trial avoidance. Panels represent individuals.

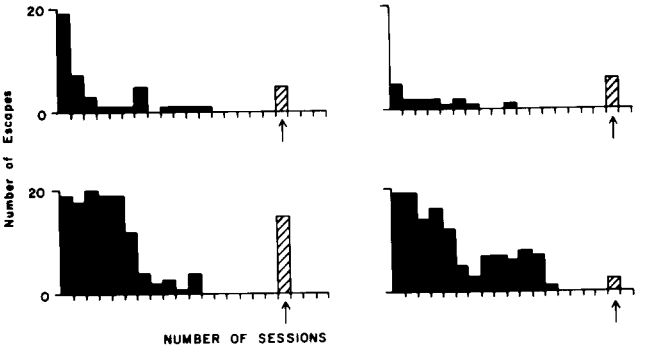

FIG. 2. Effects of 3-Carboxy-1,2,3,4-TIQ (10 mg/ml; 20 μl) on the parameter Number of Escapes, measured in Shuttle Box situation, 45 min after administration of the drug. The arrows indicate the day-session on which injection was administered. Absence of bars indicates 100% trial avoidance. Panels represent individuals.

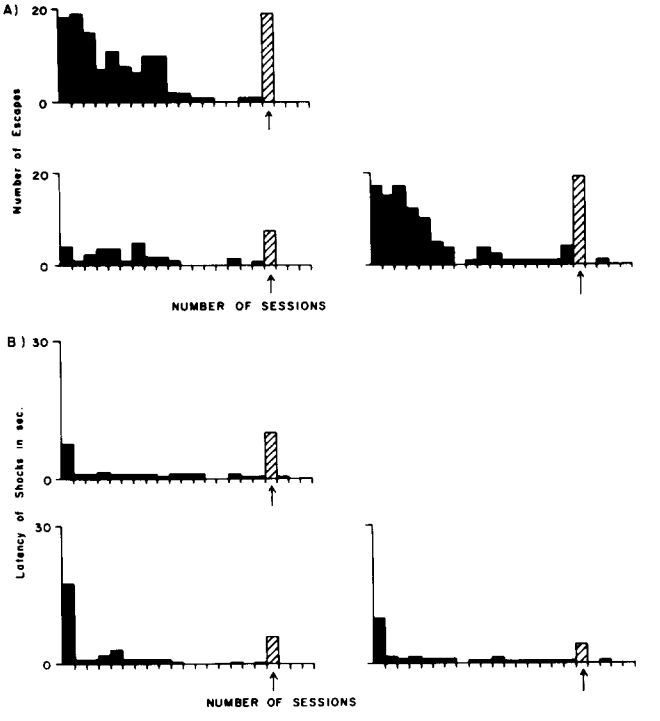

FIG. 3. Effects of 6,7-Dihydroxy-1,2,3,4-TIQ (1 mg/ml; 20 μl) on the parameters Number of Escapes (A) and Latency of Shocks (B), measured in Shuttle Box situation, 45 min after IVT administration. The arrows indicate the day-session on which drug was administered. Absence of bars indicates 100% trial avoidance. Panels represent individuals.

these parameters. Due to these results and instead of pursuing studies with drug II, we studied the behavioral alterations in the shuttle box situation.

Shuttle box. Number of escapes and latency of shocks were recorded in this situation. In the experimental session, several groups of animals received drug I (10 mg/ml; 20 μl) or saline (20 μl) and the behavioral parameters were recorded 45 min later. Differences between the controls and the experimental groups in the parameter latency of shocks ($p<0.001$; Fig. 1) were observed. Apparently, a larger number of training sessions produce an increased effect of the drug (Fig. 2) when compared to shorter sessions (not shown).

When number of escapes were recorded in different groups of animals, 90 min, or 2 hr after administration of drug I, no drug-effects were observed. Only a slight increase was detected in the latency of shocks when measured at the indicated time periods. No saline control groups were utilized in the latter series of experiments, since it was previously demonstrated that administration of the vehicle did not affect these parameters.

When drug II (6,7-Dihydroxy-1,2,3,4-TIQ) was administered at a dose of 10 mg/ml (20 μl), the effects were so violent that they led to convulsions and death of the animals. Due to this, the concentrations were lowered 10 times. Forty-five minutes after the administration of II at the new dose (1 mg/ml; 20 μl), significant effects on both, number of escapes and latency of shocks, were produced (Fig. 3). When the same parameters were recorded in a different group of animals, 2 hr after the administration of II, the behavioral effects were less pronounced (Fig. 4).

DISCUSSION

The present experiments indicate that two formaldehyde-derived TIQs, 3-Carboxy-1,2,3,4-TIQ (I), and 6,7-Dihydroxy-1,2,3,4-TIQ (II), induce behavioral alterations

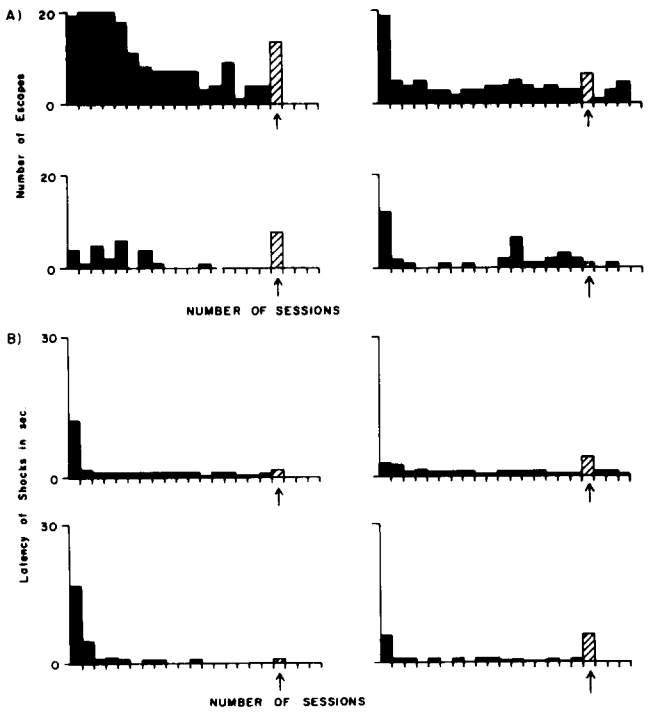

FIG. 4. Effects of 6,7-Dihydroxy-1,2,3,4-TIQ (1 mg/ml; 20 μl) on the parameters Number of Escapes (A) and Latency of Shocks (B), measured in Shuttle Box situation, 2 hr after IVT administration. The arrows indicate the day-session on which drug was administered. Absence of bars indicates 100% trial avoidance. Panels represent individuals.

in the rat 45 min after their acute administration. This finding is consistent with the previous suggestion [12] that the generation of formaldehyde within brain tissue *in vivo* could lead to the formation of TIQs with behavioral effects. The two compounds utilized as precursors are normally present in brain and could be natural precursors for the synthesis of the compounds. Particularly in the case of dopamine, its TIQ-derivative could have behavioral consequences.

When latency, activity, rearing and defecation were registered in the Open Field situation, 45 min after drug I administration, and quantitative analysis conducted, high intra- and inter-variability as well as non-consistent effects of drug or saline were detected, suggesting that this experimental situation was not adequate for quantifying the possible effects of TIQs. Despite this, as qualitative alterations were observed in the experimental groups, the Shuttle Box situation was utilized since it constitutes a more structured situation, making feasible quantification of the TIQ effects. It is important to mention that the anatomico-biological alterations that could be produced by the drug, such as body temperature, blood pressure, heart rate and/or adrenaline levels, were not controlled, obscuring further analysis of the possible mechanisms underlying the observed qualitative responses.

In the Shuttle Box situation both TIQ derivatives utilized induced behavioral alterations when recorded 45 min after IVT administration, particularly in the parameter latency of shocks (Fig. 2), in which we observed a significant increase when comparing drug-treated animals with controls and with the individual baselines. Contrary to the present study, Costall *et al.* [2] reported that 2 hr after a nialamide pretreatment (MAO inhibitor), 6,7-Dihydroxy-1,2,3,4-TIQ had no effects when measured in a time range of 9 hr after drug administration, on the behavior of the rats. The observed discrepancies could be due to the sensibility of the behavioral model utilized, shuttle box in our case and activity boxes in Costall's study, in a manner similar to our lack of quantitative differences when measuring behavioral responses in the Open Field model. Related to this, more specific models are being searched in our laboratory in order to determine at which level (i.e., sensorial, perceptual) the drug is having its effects, since at this moment we do not know accurately the nature of the observed alterations or at which level(s) the animal is affected after TIQ administration.

Regarding the possible mechanisms by which the drugs are producing these alterations we have only measured pain levels following IVT procedure with no effects on this parameter being registered.

Furthermore, differential dose-responses induced by the two compounds studied were observed. These potency differences as well as the qualitative differences in response could be explained by the fact that phenylalanine is an amino acid present generally in the nervous tissue and not necessarily related to neural transmission and behavior, whereas dopamine, and its TIQ derivative, may allow for a better possibility of interaction at the molecular level with the catecholaminergic systems involved in behavior, due to their catechol structure. Detailed pharmacological studies shall be carried out since the drug effects were reversible, as suggested by the fact that after 2 hr there were almost no alterations found on the recorded parameters, and at 24 hr the behavior of animals turned back to their original performance baseline (Figs. 1 to 4). Regarding this finding, we have not studied what happens to the compounds once they enter the brain and whether they remain for more than 45 min as TIQs and/or are catabolized into other compounds, which in turn could be responsible for the behavioral alterations observed.

In conclusion, formaldehyde-derived TIQs, formed from the reaction between formaldehyde and compounds normally present in brain tissue, are capable of inducing behavioral alterations in the rat. This finding opens the way for studies attempting to involve TIQs in the behavioral alterations present in some psychotic states in the human.

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