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Intra-Raphe Muscimol Induced Hyperactivity Depends on Ascending Serotonin Projections

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SAINATI, S. M. AND S. A. LORENS. *Intra-raphe muscimol induced hyperactivity depends on ascending serotonin projections*. PHARMAC. BIOCHEM. BEHAV. 17(5) 973–986, 1982.—Acute microinjections of the GABA agonist, muscimol (100 ng), into either the dorsal (DR) or the median (MR) raphe nucleus of etherized rats induced post-anesthesia hyperactivity as measured in photocell chambers. The increased activity counts seen after MR injections, furthermore, were 4 times greater than those following DR injections. In animals implanted with chronically indwelling cannulae, a muscimol (25–400 ng) dose-response analysis confirmed the greater sensitivity of the MR site. Subsequent experiments thus employed only MR cannulae. The benzodiazepine, chlordiazepoxide, in a subataxic dose (3.8 mg/kg, IP) by itself did not affect activity level, but enhanced the locomotor response to low doses (25–50 ng) of muscimol. Conversely, a sub-convulsant dose of the GABA antagonist, bicuculline (1.1 mg/kg, IP), completely blocked the hyperactivity produced by muscimol (50–100 ng). Bilateral electrolytic destruction of the ventral tegmental nuclei of Gudden produced hyperactivity, but failed to alter the effect of muscimol. Forebrain 5-hydroxytryptamine (5-HT, serotonin) depletion following administration of 5,7-dihydroxytryptamine did not affect baseline activity level, but markedly attenuated the locomotor response produced by intra-MR injections of muscimol. These data suggest that midbrain GABA neurons modulate activity level through a direct action on 5-HT neurons, and indicate that intra-MR muscimol induced hyperactivity depends on intact ascending 5-HT fibers.

Benzodiazepines GABA Locomotor activity Muscimol 5-Hydroxytryptamine Ventral tegmental nuclei of Gudden.

THE 1,4-benzodiazepines (BDZs) are used clinically as sedative, muscle-relaxant, anti-convulsant, and anxiolytic drugs. Not surprisingly, these compounds also produce profound behavioral effects when administered to laboratory animals. It generally is accepted that many BDZ effects are due to facilitation of the post-synaptic action of the inhibitory amino acid neurotransmitter, gamma-aminobutryic acid (GABA) [8, 9, 10, 16, 34, 37]. Relatively little is known, however, about the critical neuroanatomical sites at which a GABA-BDZ interaction leads to a specific behavioral change. There is, furthermore, a considerable body of evidence, recently reviewed [8, 47, 48], which suggests that GABA serves as a neurotransmitter for inhibitory interneurons [3, 15, 16, 17, 32, 34] within the mesencephalic B-7 and B-8 5-HT cell groups [1,12], which are the principal origins of ascending 5-HT projections to the forebrain [1,2].

In vitro autoradiographic studies have revealed that BDZ binding sites are discretely localized in the rat brain [56, 57, 58]. Using a similar technique [39], we have found moderate BDZ binding in the mesencephalic periaqueductal gray, the dorsal raphe (DR) and the median raphe (MR) nucleus [46]. The presence of BDZ receptors in the region of the DR is consistent with the electrophysiological evidence that systemic administration of benzodiazepines potentiates the suppression of spontaneous firing of DR 5-HT cells produced by iontophoretic application of the GABA agonist, muscimol [16]. Moreover, systemic chlordiazepoxide administration recently has been found to produce dose-dependent decreases in raphe unit activity in freely moving cats [43].

The interactions between midbrain benzodiazepine, GABA and 5-HT systems in the regulation of behavior largely are unexplored. Recently, however, acute local microinjections of the GABA agonist, muscimol, into the DR nuclei of ether-anesthetized rats has been found to produce a dose-dependent increase in locomotor activity, beginning 15 minutes post-injection [44]. This observation suggests that GABA-mediated inhibition of midbrain raphe neurons leads to increases in activity level. This hypothesis is supported by reports that electrolytic destruction of the MR and the ventral tegmental nuclei of Gudden (VTG) both result in hyperactivity as measured in the open field [26, 28, 29]. On the other hand, 5,7-DHT lesions which deplete forebrain 5-HT fail to affect open-field activity [20,28]. In another report, 5,7-DHT lesions were found to produce increases in

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24-hour tilt-cage activity. These rats treated with 5,7-DHT were, nonetheless, hypoactive when tested in the open field [30].

In the present study, we first replicated the findings that acute microinjections of muscimol into the DR produce hyperactivity [44]. Furthermore, we found that acute microinjections of muscimol (100 ng) into the MR produce increases in activity which were 4 times greater than those seen after DR injections. A dose-dependent analysis using animals with chronically-indwelling cannulae showed that the MR, indeed, was more sensitive to the effects of muscimol. Thus, subsequent experiments utilized only cannulae chronically indwelling in the MR. To determine whether or not the hyperactivity effects of muscimol were due to activation of GABA receptors, we attempted to potentiate the effects of intra-MR muscimol by administering a benzodiazepine, chlordiazepoxide (CDP), and to block the effect with a GABA antagonist, bicuculline. Since destruction of the VTG produces hyperactivity, we attempted to determine what effect this manipulation would have on the muscimol dose-response relationship. Finally, to ascertain the role of the ascending 5-HT systems in maintaining the locomotor response to muscimol, we destroyed these projections with 5,7-DHT and repeated the muscimol dose-response analysis.

GENERAL METHOD

Animals

The experimental subjects were male Sprague-Dawley rats (King Animal Farms, Orange, WI), 90–120 days old and weighing 310–375 g at the time of surgery. The animals were housed individually in a temperature (22±1°C), humidity (40–52%), and illumination (12 hour light-dark cycle) controlled room. Food and water were available ad lib in the home cage.

Drugs

Intraperitoneal administration. Chlordiazepoxide hydrochloride (Roche, Nutley, NJ) was dissolved in 0.9% saline to a final concentration (as the base) of 3.8 mg/ml. (+)−Bicuculline (Sigma Chemical Co., St. Louis, MO) was dissolved in acidified vehicle (pH=5.5–6.0) to a final concentrations of 1.1 mg/ml. Desmethylimipramine hydrochloride (Merrell, Cincinnati, OH) was dissolved in 0.9% saline to a concentration (as the salt) of 20 mg/ml.

Intracranial administration. Muscimol (Sigma) was dissolved in 0.9% saline in concentrations of 25, 50, 100, 200 and 400 ng/0.5 μl.

Surgery

A Kopf stereotaxic instrument was used. The incisor bar was set 3.2 mm above the interaural plane. At the time of surgery, each animal received 50 mg/kg ampicillin (Omnipen-N®, Wyeth, Philadelphia, PA) and 0.4 mg/kg atropine sulfate (Lilly, Indianapolis, IN), intramuscularly, and chloramphenicol (Chloromycetin®, 1% opthalmic ointment; Parke-Davis, Morris Plains, NJ) topically around the wound. For the acute intra-raphe microinjections, ether (Mallinckrodt, McGaw Park, IL) was employed as an anesthetic, and the wound margins were infiltrated with 0.1 ml of 1% lidocaine hydrochloride (Xylocaine®, Astra, Worcester, MA) in order to minimize post-operative pain. For other surgical procedures intraperitoneal pentobarbital sodium (50 mg/kg; Butler, Columbus, OH) was used as an anesthetic. All wounds were closed with autoclips (Clay Adams, McGaw Park, IL).

Acute intra-raphe injections. Muscimol (100 ng in 0.5 μl of vehicle) or saline (0.5 μl) was administered via a 5.0 μl Hamilton syringe oriented mid-sagittally at an angle of 47 degrees caudal to the vertical plane. The solution was delivered slowly (in about 60 seconds). The needle was left in situ for 2 min following completion of the injection. Coordinates for the dorsal raphe placement were 6.2 mm caudal and 9.6 mm ventral to the skull surface 1.0 mm rostral to lambda (L +1). Coordinates for the median raphe placement were 8.1 mm caudal and 12.1 mm ventral to L +1.

Chronic intra-raphe cannula placement. A guide cannula (0.46 mm o.d. and 0.25 mm i.d.; Plastic Products Co., Roanoke, VA) was implanted in either the dorsal or the median raphe nucleus. A stylet (0.23 mm dia.) then was inserted such that its tip was flush with that of the guide cannula. This assembly then was cemented onto stainless steel anchoring screws embedded in the skull. The coordinates were the same as mentioned above.

Electrolytic lesions. A 0.25 mm diameter stainless steel wire insulated with Expoylite except at the cross section of its tip was used as the lesion electrode. This electrode was connected to the positive pole of a Grass model DCLM-5A direct current lesion maker (Grass Medical Instruments Co., Quincy, MA). An alligator clip attached to the wound margin served as the ground. Lesions were produced bilaterally in the ventral tegmental nuclei of Gudden (VTG) by passing 2.0 milliamperes direct current for 2 seconds via the anode. The anode was inserted stereotactically at an angle 47 degrees caudal to the vertical plane. The coordinates used were 0.5 mm lateral to the midsagittal suture, and 7.5 mm caudal and 11.7 mm ventral to the skull surface at L +1.

Neurotoxic lesions. The specific serotonin neurotoxin, 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT; Sigma) [4], was dissolved in 0.2% ascorbate in 0.9% saline, and was injected bilaterally either into the lateral cerebral ventricles (75 μg free base in 5.0 μl vehicle), or into the ventromedial mesencephalic tegmentum (4.0 μg base in 2.0 μl) at a rate of 0.5 μl/min. The coordinates used were 0.5 mm rostral to bregma, 1.2 mm lateral to the midline, and 5.5 mm ventral to the skull surface for the lateral ventricle injections; and, an angle of 47 degrees caudal to the vertical plane, 5.5 mm caudal to lambda, 0.6 mm lateral to the midline, and 11.0 mm ventral to the reading at lambda for the mesencephalic tegmentum injections. These subjects all received desmethylimipramine hydrochloride, 20 mg/kg intraperitoneally, 30–45 minutes prior to surgery to prevent uptake of 5,7-DHT into noradrenergic nerve terminals [4]. Because desmethylimipramine potentiates the anesthetic effects of pentobarbital, the dose of the anesthetic for these animals was 35 mg/kg.

Apparatus

Activity level was measured in enclosed cylindrical photocell chambers (46 cm dia. × 42 cm high; Model No. PAC-001, Lehigh Valley Electronics, Inc., Beltsville, MD) with wire mesh floors. The interiors of the walls and covers of these chambers were painted flat black. Interruption by the animal of any one of six photocell beams located at the base of the chamber activated an electromechanical counter.

Biochemical Analysis

The animals were killed by decapitation and their brains

quickly removed and dissected on a glass plate over dry ice as described previously [27]. The hippocampi and striata were wrapped in aluminum foil, flash frozen in liquid nitrogen, and stored in a −70° C freezer for no more than 3 weeks prior to assay. The brainstems were placed in phosphate-buffered formalin and fixed for at least 2 weeks prior to sectioning.

5-Hydroxytryptamine (5-HT) and 5-hydroxy-3-indoleacetic acid (5-HIAA) levels were determined by high-performance liquid chromatography (HPLC) [33]. Each tissue sample was sonicated for 20 sec in 500 μl of 0.1 N perchloric acid which contained 1.0 mM sodium EDTA, 0.3 mM thioglyoxylic acid, and 50 μg of the internal standard, N-methyl-5-hydroxytryptamine (NM5HT). The homogenate was centrifuged at 15,000 × g for 10 min, then microfiltered by centrifugation using millipore tubes. The filtrate was drawn into a six port rotary valve (Rheodyne Model No. 7125) and injected into an HPLC system (BioAnalytical Systems, Inc., West Lafayette, IN) utilizing a reverse phase column (10 μm μ-Bondapak C-18, Waters Associates, Milford, MA) and a Waters Model No. M-45 pump. The mobile phase consisted of 0.15 M monochloroacetic acid, 10 mM octanylsulfonic acid and 1.0 mM EDTA. The flow rate was 1.5 ml/min. Electrochemical detection was accomplished with an LC-4 amperometer (BioAnalytical Systems). Retention times were: 5-HIAA—24.8 min, 5-HT—29.8 min, and NM5HT—32.3 min.

Histology

Those animals not requiring biochemical determinations of 5-HT and 5-HIAA levels were perfused transcardially with 100 ml of saline followed by 100 ml of phosphate-buffered formalin. Their brains then were removed and post-fixed for at least one week prior to sectioning. After the tissue was well fixed, it was transferred to a solution containing 5% sucrose in 0.1 M phosphate buffer for 24–48 hours. The tissue then was frozen with dry ice and cut on a sliding microtome. Every fourth section (50 μm) was retained, mounted on a gelatin coated slide and stained using the cresylecht violet procedure [42].

Statistical Analysis

The data from Experiment 2 were analyzed using a complex Latin square design. Data from Experiment 3 were analyzed with an analysis of variance (ANOVA) with a repeated measures, two factors design. The data from the biochemical and the remaining behavioral experiments were analyzed by an ANOVA, two-factor mixed design with repeated measures on one factor; and, a three-factor mixed design with repeated measures on two factors [6,23]. Individual between-group comparisons were performed, when merited, by Newman-Keuls' multiple range test [6, 22, 35], or by an F-test for simple effects [6]. For within group comparisons, a Newman-Keuls test for related measures was used [6,22]. Statistical analyses were performed on a Hewlett-Packard HP-85 minicomputer with a No. 90053 statistical program package, as well as with Nos. 300-0027, 300-0029, 300-0035 and 300-0043 ANOVA programs (Hewlitt-Packard Corp., Corvallis, OR).

RESULTS

EXPERIMENT 1: ACUTE INTRA-MIDBRAIN MICROINJECTIONS OF MUSCIMOL

It has been reported [44] that acute injections of muscimol (50 and 100 ng) into the dorsal raphe (DR) nucleus of rats produce elevations in locomotor activity. In our first experiment, we attempted to replicate these findings. In addition, the effects of muscimol injections into the DR were compared to those following injections into the median raphe nucleus (MR).

Procedure

Rats were anesthetized with ether and injected with muscimol (100 ng in 0.5 μl of vehicle) or saline (0.5 μl) into either the DR or the MR as described in the General Method section. Four additional animals served as sham-operated controls. These animals were treated in the same manner as the injected rats, except that a needle was not lowered into their brains. Fifteen minutes post-injection, the rats, fully awake, were placed in the photocell chambers, and their activity counts recorded every 15 minutes for 90 minutes.

Results

Histological analysis. Of the 7 animals which received muscimol injections into the MR, one was eliminated from the study prior to analysis of the behavioral data because the needle tract terminated 1.5 mm laterally in the mesencephalic reticular formation. Of the remaining animals, the needle tips in 4 terminated in the rostral extent of the B-8 5-HT cell group [12, 25, 49] just dorsal to the rostral one-third of the interpenduncular nucleus. The needle tips in the other 2 rats were localized in the MR at the level of the ventral tegmental nucleus of Gudden (Fig. 1). The needle tips of the 3 animals which received vehicle injections into the MR were placed similarly.

Of the 7 animals which received muscimol injections into the DR, one was rejected from further analysis because the needle tip was localized within the lumen of the cerebral aqueduct. In the remaining animals, the needle tips terminated in the rostral DR at the level of the third and fourth cranial nerve nuclei (Fig. 2). The animals which received vehicle injections into the DR had similar needle placements.

Activity level. An ANOVA of the number of counts per 15 minutes failed to demonstrate any significant differences between the 4 sham-operated, the 3 MR-vehicle injected, and the 3 DR-vehicle injected animals. These groups, therefore, were combined into a single control group. Subsequently, an ANOVA revealed significant treatment, $F(2,20)=23.3$, $p<0.001$, time, $F(14,140)=13.4$, $p<0.001$, and interaction, $F(14,140)=7.6$, $p<0.001$, effects between the control and muscimol injected groups. The cumulative activity scores per 15 minutes for each group are shown in Fig. 3.

Between-group comparisons of the total 90 minute activity scores showed that both the MR- and DR-muscimol injected groups were significantly more active than the control group. The MR-muscimol injected group, furthermore, was significantly more active than the DR-muscimol injected group.

Individual group comparisons of the activity scores for each 15 minute period (Fig. 3) revealed that the MR-muscimol injected group was significantly more active during the first 15 minutes in the apparatus and throughout the remainder of the 90 minute test session. The activity level of the DR-muscimol injected group did not differ from control during the first 15 minute segment, but was significantly higher than control for the remainder of the test session. The hyperactivity induced by muscimol injections into the MR, thus, was more rapid in onset and of a greater magnitude,

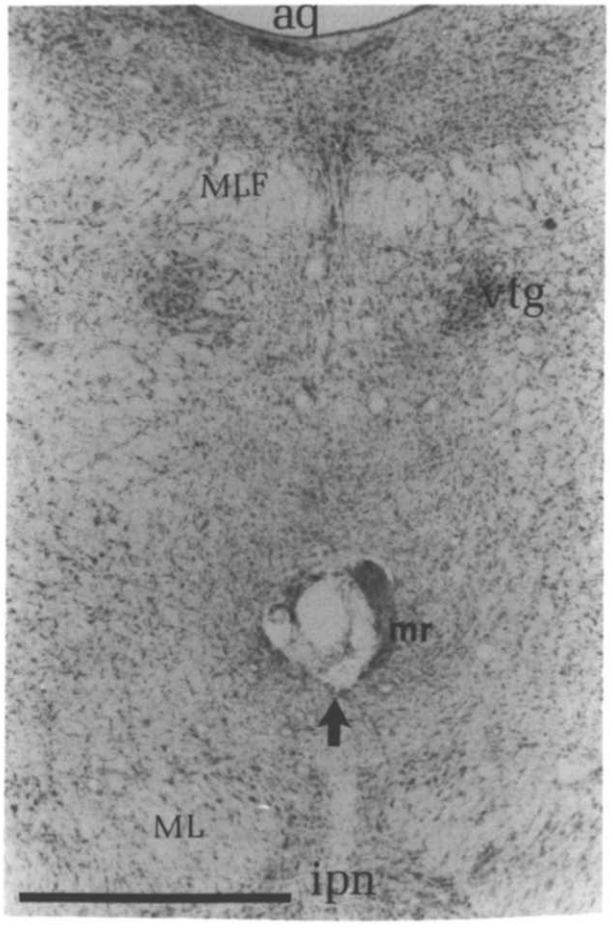

FIG. 1. Photomicrograph of median raphe (MR) needle tip. Cresylecht violet stained coronal section (50 μm) through the caudal midbrain of a MR-muscimol injected rat from Experiment 1. The needle tip (black arrow) appears as a small cavity rimmed by a glial scar, and, in this animal, terminates in the caudal portion of the B-8 5-HT cell group at the level of the VTG. Bar represents 1 mm. Abbreviations: Aq=cerebral aqueduct of Sylvius; IPN=interpenduncular nucleus; ML=median lemniscus; MLF=medial longitudinal fasciculus; VTG=ventral tegmental nucleus of Gudden.

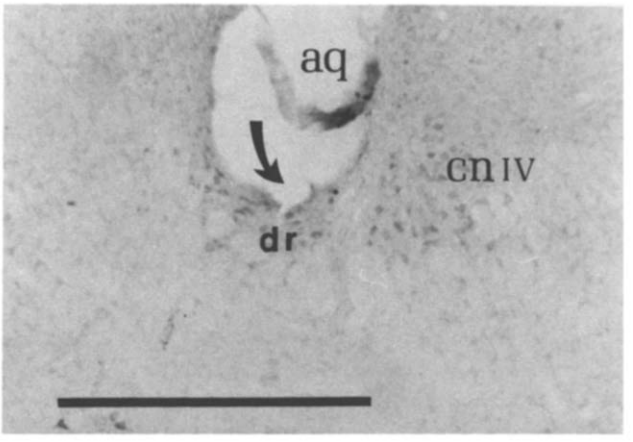

FIG. 2. Photomicrograph of dorsal raphe (DR) needle tip. Nissl stained coronal section (50 μm) through the midbrain of a DR-muscimol injected rat from Experiment 1. The needle tip (black arrow) appears as a small cavity rimmed by a glial reaction, and, in this animal, terminates in the rostral part of the B-7 5-HT cell group at the level of cn IV. Bar represents 1 mm. Abbreviations: Aq=cerebral aqueduct of Sylvius; cn IV=trochlear nucleus.

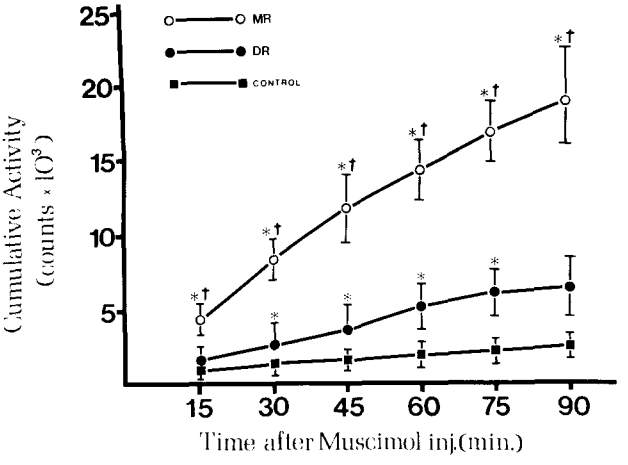

FIG. 3. Activity scores after acute muscimol injection. Group (Mean±S.E.M.) cumulative activity scores in rats following acute microinjection of vehicle (control, n=10) or muscimol into the dorsal (DR, n=6) or median (MR, n=6) raphe nucleus. *Significantly different from control group ($p<0.01$); †significantly different from DR-muscimol injected group ($p<0.01$, Newman-Keuls' multiple range test).

than that produced by the DR-muscimol injections. In fact, at the end of the 90 minute session, the cumulative activity score of the MR-muscimol injected group was approximately four times greater than that of the DR-muscimol injected group.

Conclusion

In the first experiment we were able to confirm the previous report [44] that muscimol injections into the DR produce hyperactivity. In addition, we found that muscimol injections into the MR also produced a hyperkinetic effect, and, that this effect not only appears earlier after injection, but is of a much greater magnitude.

EXPERIMENT 2: MUSCIMOL DOSE-RESPONSE RELATIONSHIP IN ANIMALS WITH CHRONICALLY INDWELLING CANNULAE

In order to rule out the possible interactions of the ether anesthesia and the acute surgical trauma with the effects of intra-midbrain muscimol injections on locomotor activity, we performed a dose-response analysis using animals having cannulae chronically implanted in the DR or the MR. Furthermore, since each animal served as its own control and received each drug dose, a complex Latin square design was employed in order to determine whether a given dose schedule produced effects significantly different from another.

Procedure

Beginning 1 week post-operatively, the animals were

adapted to the test apparatus and injection procedure [21] for 3 consecutive days (Wednesday–Friday). The subjects were placed in the photocell chambers for 30 minutes, removed and wrapped in a towel for 30–60 seconds, then returned to the chambers for an additional 2 hours. During the subsequent three weeks, the animals were tested Monday through Friday, muscimol or vehicle injections being performed on Tuesdays and Thursdays. The animals were placed in the apparatus for 30 minutes and their activity scores recorded for each 15 minute segment. The rats then were removed, wrapped in a towel, and injected (on drug days only) over 30 seconds with 0.5 μl of drug solution. The animals then were placed back in the chambers for an additional 2 hours. Activity counts were recorded 15, 30, 60, 90 and 120 minutes post-injection. Muscimol (0, 25, 50, 100, 200 and 400 ng in 0.5 μl saline) was injected into the MR or the DR according to a complex Latin square design [6] with at least 10 animals receiving each of the 6 dose sequences.

Results

Histological analysis. Prior to performing any statistical analyses on the behavioral data, we screened the cannula placements. Of the 60 animals implanted with MR cannulae, 30 were found acceptable. The cannulae in these animals terminated within the B-8 5-HT cell group coinciding with the caudal linear and median raphe nuclei, just dorsal to the interpeduncular nucleus. The histological appearance of the cannula placements in these animals was similar to the needle tracts in Experiment 1 (see Fig. 1). The cannula tips in the rats rejected from the study terminated at least 1.5 mm lateral, dorsal, and/or caudal to the B-8 5-HT perikarya.

Of the 72 rats which were implanted with DR cannulae, only 30 had acceptable placements. The cannula tips in these animals all were localized within the boundaries of the DR (B-7 5-HT cell group). The cannulae in the animals eliminated from the study terminated at least 0.75 mm dorsal and/or lateral to the DR. The histological appearance of the DR cannula tracts was indistinguishable from that of the DR needle tracts in Experiment 1 (see Fig. 2).

Activity level. An ANOVA of the activity scores per 15 minutes for the 30 minute pre-drug session on each of the 6 drug days showed that there were no significant cannula placement, days, or interaction effects. There was a significant time effect, $F(1,58)=63.20$, $p<0.001$, due to the greater activity of the animals during the first 15 minutes of the 30 minute pre-drug period. These results show that the animals' activity levels were comparable during the pre-drug sessions on each of the 6 drug days. Subsequently, a complex Latin square analysis of the total 120 minute post-injection activity scores was performed. This analysis revealed a significant overall effect of placement site, $F(1,48)=5.04$, $p<0.05$, a significant overall effect of the muscimol dose, $F(5,240)=29.08$, $p<0.0001$, and a significant interaction between dose and injection site, $F(5,240)=22.21$, $p<0.0001$. Importantly, the effects of order of treatment were not significant.

As shown in Fig. 4, injections of muscimol (50–400 ng) into the MR produced significant increases in activity. The peak effect was seen after the 100 ng dose. In contrast, only the two highest doses of muscimol (200 and 400 ng) induced hyperactivity following injection into the DR. (200 ng). The muscimol-induced hyperactivity was most prominent during the first 30 minutes post-injection, and diminished in magnitude over the subsequent 90 minutes.

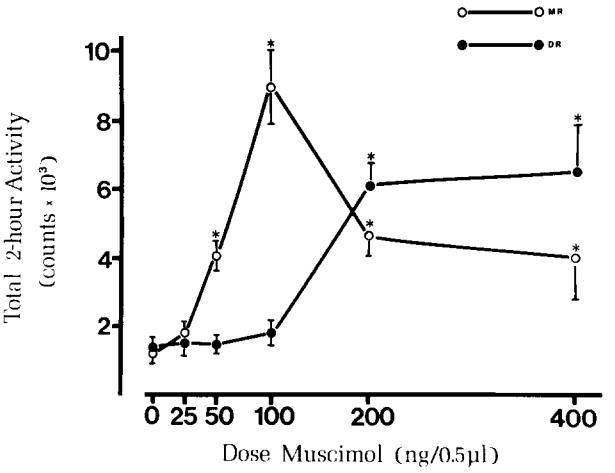

FIG. 4. Dose-activity effects of intra-raphe muscimol. Total activity scores (Mean±S.E.M.) for the 2 hour period following muscimol injections via chronically indwelling median (MR, n=30) or dorsal (DR, n=30) raphe cannulae. The muscimol was administered according to a Latin square design with 5 animals receiving each of the dose sequences. *Significantly higher than control condition ($p<0.01$, Newman-Keuls' test for related means).

Conclusion

These observations indicate that injection of muscimol into the MR produces a significant elevation in activity level at a dose (50 ng) one-fourth that required to produce a similar effect when injected into the DR. The magnitude of the hyperkinetic effect following the injection of an optimal dose of muscimol into the MR (100 ng), moreover, is at least 1.5 times greater than after such an injection into the DR (200–400 ng). These results suggest the possibility that the hyperkinetic effect of muscimol following injection into the DR may be due to diffusion of the drug to an effective MR site.

Inasmuch as the MR proved to be more sensitive to muscimol than the DR, we decided to concentrate our subsequent efforts on the MR site. Furthermore, since we found in Experiment 2 that the order of administration of the drug treatments did not significantly affect the experimental outcome, we also decided to employ the more cost-effective randomized block design [23] in our subsequent experiments.

EXPERIMENT 3: INTERACTIONS OF PERIPHERAL BICUCULLINE AND CHLORDIAZEPOXIDE WITH INTRA-RAPHE INJECTIONS OF MUSCIMOL

In the previous two experiments it was shown that intraraphe injections of muscimol produce hyperactivity as measured in photocell chambers. If the hyperkinetic effect of muscimol is due to the activation of GABA receptors, then this effect should be attenuated by the peripheral administration of the GABA receptor antagonist, bicuculline, and potentiated by the facilitator of GABA-ergic neurotransmission, chlordiazepoxide. It was the objective of the present experiment to test these hypotheses.

In a preliminary study [45] we determined that chlordiazepoxide, when administered intraperitoneally in doses greater than 3.8 mg/kg produced decreases in locomotor activity as measured in dark photocell chambers. Following

these doses, the animals appeared ataxic and sedated. Doses ranging between 1.2–3.8 mg/kg did not affect activity as compared to control. We also found that intraperitoneal injection of bicuculline in doses ranging between 0.1–1.1 mg/kg had no effect on activity level. Higher doses regularly produced seizures, the convulsant dose for 50 percent of the cases (ED-50) being 2.2 mg/kg. In the following experiment, therefore, the 3.8 mg/kg dose of chlordiazepoxide, and the 1.1 mg/kg dose of bicuculline were used.

Procedure

Cannulae were implanted in the MR of 18 animals as described in the General Method section. Nine rats were assigned to the chlordiazepoxide-muscimol interaction study, and 9 to the bicuculline-muscimol interaction study. The testing procedure was identical to that described in Experiment 2, except that the activity counts were recorded every 15 minutes for only one hour post-injection.

Fifteen minutes prior to receiving muscimol (0, 25, or 50 ng) injections into the MR, one group of rats received either saline (1.0 ml/kg) or chlordiazepoxide (3.8 mg/kg) intraperitoneally. Fifteen minutes prior to receiving intra-raphe muscimol (0, 50, or 100 ng), the other groups of rats received either the acidified vehicle (1.0 ml/kg) or (+)−bicuculline (1.1 mg/kg) intraperitoneally.

A repeated measures—two factors ANOVA design was used in the statistical analysis of the behavioral data. This is an analysis which can be used when all treatments in a two-factor experiment are administered to each subject [6]. The effects of each treatment can be discerned individually, and the interaction between the two can be ascertained. Because no statistical test for simple effects exists specifically for the repeated measures—two factor ANOVA design, individual comparisons between means were made with Student's *t*-test for related measures [6,50].

Results

Histological analysis. Three rats were eliminated from the study prior to statistical analysis of the behavioral data, since the cannula placements in these animals were localized 1.5 mm lateral to the MR. The cannula tips in the accepted animals terminated in the B-8 5-HT cell group dorsal to the interpeduncular nucleus (7 in the chlordiazepoxide-muscimol interaction study and 8 in the other study).

Locomotor activity. Chlordiazepoxide-muscimol interaction: An ANOVA of the total 60 minute post-injection activity scores failed to demonstrate a significant effect of chlordiazepoxide alone. The effect of muscimol, however, was significant, $F(2,16)=7.37$, $p<0.01$, as was the muscimol-chlordiazepoxide interaction, $F(2,16)=6.30$, $p<0.01$. Thus, as shown in Fig. 5, the parenteral injection of chlordiazepoxide potentiated the effect of intra-raphe muscimol (25 and 50 ng) on locomotor activity.

Bicuculline-muscimol interaction: An ANOVA of the total 60-minute post-injection activity scores failed to show a significant effect of bicuculline by itself. However, the effect of muscimol was significant, $F(2,16)=8.64$, $p<0.005$, as was the bicuculline-muscimol interaction, $F(2,16)=6.69$, $p<0.05$. Figure 6 depicts the blockade of the hyperkinetic effect of intra-raphe muscimol (50 and 100 ng) by the peripheral administration of bicuculline.

Conclusion

These results support the view that the hyperkinetic effect of intra-raphe muscimol injection is due to an activation of GABA receptors.

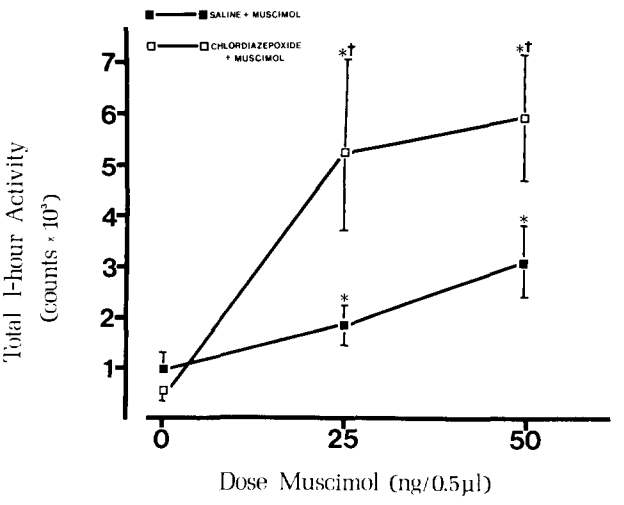

FIG. 5. Chlordiazepoxide potentiates the muscimol effect. Total activity scores (Mean±S.E.M.) for the one hour period following the injection of muscimol (0, 25 or 50 ng) into the median raphe nucleus (n=7). Fifteen minutes prior to receiving muscimol, the animals received either chlordiazepoxide (3.8 mg/kg) or saline (1.0 ml/kg) intraperitoneally. *Significantly greater than the corresponding control condition ($p<0.01$); †significantly higher than the corresponding saline plus muscimol effect ($p<0.05$, Students *t*-test, 2-tailed).

EXPERIMENT 4: MUSCIMOL DOSE-RESPONSE CURVE IN ANIMALS WITH LESIONS OF THE VENTRAL TEGMENTAL NUCLEI OF GUDDEN

In vitro autoradiographic studies have demonstrated that whereas the DR and MR are sites of only moderate benzodiazepine binding, the ventral tegmental nuclei of Gudden (VTG) show extremely dense benzodiazepine labelling [57,58]. Since the benzodiazepines are thought to bind allosterically to a membrane complex including the GABA receptor, there is a correlation between the loci of benzodiazepine and GABA binding sites. The VTG, in contrast to the DR and MR, are devoid of 5-HT perikarya [25,49]. Bilateral electrolytic lesions of the VTG results in increased open field locomotor activity [29]. In contrast, 5,7-dihydroxytryptamine lesions of the ascending serotonin pathways do not affect activity level in the open field [26,28]. Since the VTG lie immediately dorsolateral to the MR, it is possible that the locomotor effects of muscimol injections into the MR may be due to diffusion to the VTG. Activation of GABA receptors located on VTG neurons could result in hyperpolarization and suppressed rates of firing. This transient inhibition, or "functional lesion," could lead to locomotor hyperactivity. We, therefore, ablated the VTG electrolytically in order to determine whether or not this manipulation would affect the dose-response relationship for muscimol.

Procedure

Three groups of animals were employed. One group was anesthetized, the scalps incised and retracted, and craniotomies performed. The lesion electrode was not low-

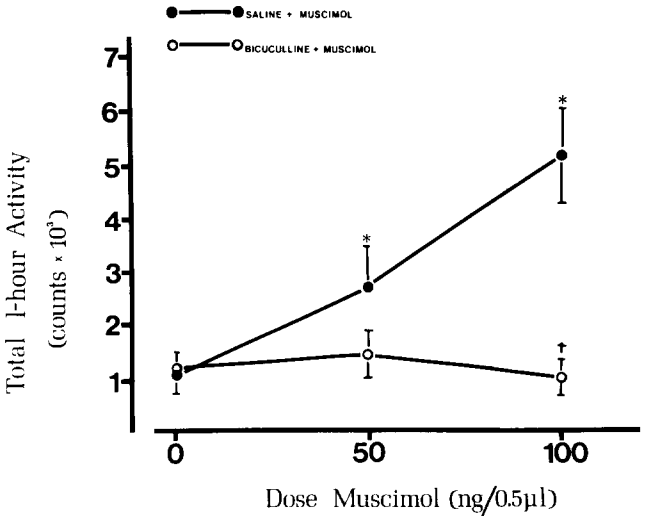

FIG. 6. Bicuculline blocks the muscimol effect. Total activity scores (Mean±S.E.M.) for the one hour period following injection of muscimol (0, 50 or 100 ng) into the median raphe nucleus (n=8). Fifteen minutes prior to receiving muscimol the animals received either bicuculline (1.1 mg/kg) or acidified vehicle (1.0 ml/kg) intraperitoneally. *Significantly higher than corresponding vehicle-injected control condition ($p<0.01$); †significantly lower than corresponding vehicle plus muscimol effect ($p<0.002$, Student's t-test, 2-tailed).

ered into the brain, nor were intracranial cannulae implanted. This sham-operated control group consisted of 8 animals. In the other 2 groups, electrolytic VTG lesions (n=15) were produced or sham-operations (n=8) were performed first, and immediately thereafter, MR cannulae were implanted as described in the Materials and Methods section. Behavioral testing began 2 weeks later.

Since bilateral electrolytic destruction of the VTG has been reported to produce hyperactivity in the open field and to facilitate acquisition of a two-way (shuttlebox) conditioned avoidance task [28,29], these two tests were used to screen the animals behaviorally for effective VTG lesions prior to the initiation of the intra-raphe muscimol study. Thus, only lesion animals with both open field activity scores and total avoidance responses above control range subsequently were tested for a muscimol dose-response relationship in the photocell activity cages. In addition, the locus and effectiveness of the VTG lesions were assessed histologically and biochemically as described previously [29].

Open field activity. Open field activity was tested in a 100×100 cm arena with 40 cm high walls. Illumination was provided by a 15-W bulb centered 120 cm above the arena. The floor of the open field was painted white and divided by black lines into 25 equal squares. Animals naive to the apparatus were placed in the center square at the start of the test. Crossings from square to square with all four limbs were recorded by the experimenter at 3 minute intervals for 9 minutes.

Two-way conditioned avoidance. Rats were trained to avoid footshock in a 100 cm long, 24 cm wide and 30 cm high shuttlebox illuminated with a 40-watt fluorescent tube mounted outside the rear wall. The front wall was composed of clear Plexiglas, while the remaining surfaces were of translucent white Plexiglas. The test chamber was divided by a 7.5 cm high metal hurdle into two equal sized compartments. Each side of the chamber contained a grid floor connected by a selector switch to a Grayson-Stadler model E-1064-GS shocker and scrambler. A 4 ohm speaker was mounted in each wall for the delivery of a 75 decibel white noise conditioned stimulus (CS). The ambient noise level approximated 50–55 decibels.

Acquisition of the two-way avoidance response was examined beginning 7 days after completion of open field testing. A single session of 50 massed trials was given to each VTG lesion animal which had been hyperactive in the open field, and to each cannulated and non-cannulated control animal. The first training trial started 3 minutes after the rat was placed in one of the compartments. The intertrial interval was 30–45 seconds. The CS was terminated when the rat crossed into the opposite compartment. The unconditioned stimulus, a 0.8 milliampere continuous footshock, was administered if the animals failed to cross the barrier within 5 seconds after CS onset. Intertrial barrier crossings were recorded, but not punished. Escape and avoidance latencies were measured with a stopwatch.

Muscimol dose-response analysis. Beginning 1 week after completion of two-way avoidance testing, the animals were tested for locomotor activity in the photocell chambers using a procedure identical to that described in Experiment 2, with the exception that activity levels were recorded every 15 minutes for only one hour post-injection. The doses of muscimol employed were 0, 50, 100 and 200 ng.

Results

Histological analysis. Of the 15 animals on which both VTG lesions and MR cannulations were performed, only 10 met the behavioral criteria for effective VTG lesions and were included in the drug study. Of these, only 6 had well placed cannulae in the B-8 5-HT cell group dorsal to the interpeduncular nucleus (Fig. 7) and subsequently were analyzed behaviorally and biochemically. Since the cannula placements in the 8 control animals were similar to those in the accepted lesion subjects, none of these animals was rejected from the study.

Biochemical analysis. The striatal and hippocampal concentrations of 5-HT and its metabolite, 5-HIAA, were assayed by HPLC. The following groups were compared: the accepted VTG lesion plus MR cannula animals (n=6), the sham-operated plus MR cannula animals (n=8), and the sham-operated non-cannulated controls (n=8). As shown in Table 1, hippocampal 5-HT levels in the VTG lesion animals were significantly reduced in comparison with both the sham-operated controls (57%) and the MR cannulae implanted controls (52%). The hippocampal 5-HIAA levels in the VTG lesion rats showed similar reductions. In contrast, the striatal 5-HT concentrations of the 3 groups did not differ significantly. The striatal 5-HIAA level in the VTG lesion rats, however, was significantly lower (18%) than control. These observations are in agreement with those previously reported [29]. Importantly, the MR cannula placements by themselves did not significantly affect the concentration of 5-HT in either the hippocampus of the striatum. Although the hippocampal 5-HIAA level of the sham-operated MR cannulated rats was slightly but significantly lower than control (24%), these data suggest that the MR cannulae did not significantly damage the 5-HT perikarya comprising, or the 5-HT fibers emanating from, the B-8 5-HT cell group.

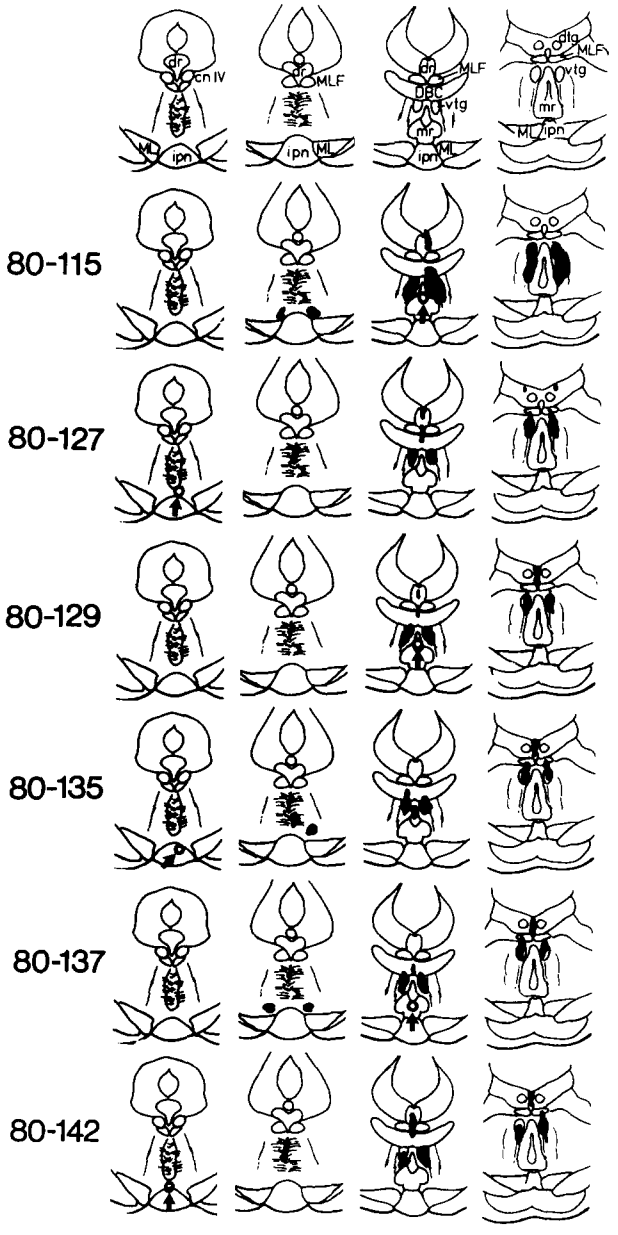

FIG. 7. VTG lesion and MR cannula placement sites. Reconstruction of damage (blackened area) in the VTG lesion rats. Black circles with arrows indicate loci of cannula tips. Numbers identify individual animals. Abbreviations: cnIV=trochlear nucleus, dr=dorsal raphe nucleus, dtg=dorsal tegmental nucleus of Gudden, ipn=interpeduncular nucleus, mr=median raphe nucleus, vtg=ventral tegmental nucleus of Gudden; DBC=decussation of brachium conjunctivum, ML=medial lemniscus, MLF=medial longitudinal fasciculus.

Behavioral analysis. Open field activity and conditioned avoidance acquitision: These two behavioral tests were used to screen the effectiveness of the VTG lesions. Thus, only the ranges of scores obtained from the rats accepted for the intra-raphe muscimol dose-response analysis are presented. The ranges of total 9-minute open field activity scores were as follows: MR cannula implanted plus VTG lesion group (n=6), 365–521; sham-operated MR cannula implanted group (n=8), 128–298; sham-operated non-cannulated group (n=8), 52–235. The ranges for the total number of conditioned avoidance responses emitted during the 50 massed trials were: VTG lesion plus MR cannula implanted animals, 28–43; sham-operated MR cannula implanted rats, 9–27; sham-operated controls, 1–26. The VTG lesion plus MR cannula group also tended to show more inter-trial spontaneous barrier crossings (2–248) during the entire test session (about 45 minutes in length) than either the non-lesion MR cannula implanted (0–22) or the sham-operated non-cannulated control (0–14) groups.

Muscimol dose-response analysis: An ANOVA (three factor mixed design-repeated measures on two factors) [6] of the 30 minute pre-injection activity levels of the VTG lesion plus MR cannula, versus the non-lesion MR cannula implanted group for the 4 drug days revealed both significant group, $F(1,10)=30.66$, $p<0.0005$, and time, $F(1,10)=112.44$, $p<0.0001$, effects. This latter effect was due to the greater level of activity of the animals during the first 15 minutes in the apparatus.

A subsequent ANOVA of the total 60 minute post-injection activity scores for the 4 drug days showed neither significant group nor interaction effects. As expected, the dose effects were significant, $F(3,30)=11.86$, $p<0.0001$. Muscimol produced similar dose-dependent elevations in activity level in both groups (Fig. 8). Because the VTG lesion animals evidenced higher baseline levels of activity than the non-lesion subjects, we reexamined the post-muscimol effects after taking this difference into consideration. This was done by subtracting the activity scores obtained by each rat during the last 15 minutes of the pre-drug control period from each of its four 15-minute post-injection scores. An ANOVA of these data, however, also failed to demonstrate any significant difference between the VTG lesion and control groups. As expected, the muscimol dose effect was significant, $F(3,30)=16.68$, $p<0.0001$, but the group-dose interaction was not.

Conclusion

These data suggest that the GABA and benzodiazepine receptors located on neuronal cell bodies or fibers within the VTG do not mediate the hyperkinetic effects of intra-raphe muscimol administration.

EXPERIMENT 5: MUSCIMOL DOSE-RESPONSE CURVE IN ANIMALS WITH LESIONS OF THE ASCENDING 5-HT PROJECTIONS

We have found benzodiazepine binding sites in the MR, although not as dense as in the VTG [46]. Since destruction of the VTG had no effect on the locomotor response to intraraphe muscimol, and since the peripheral administration of benzodiazepines has been reported to affect the metabolism of 5-HT, we decided to determine the effect of forebrain 5-HT depletion on the effects of intra-raphe muscimol.

Procedure

The ascending 5-HT projections were destroyed by injecting 5,7-dihydroxytryptamine (5,7-DHT) intracerebroventricularly (n=12) or intramesencephalically (n=12) as described in the Materials and Methods section. These animals, as well as vehicle-injected controls, were implanted with MR cannulae during the same operative session. Beginning 2 weeks post-operatively the animals were tested as

TABLE 1

STRIATAL AND HIPPOCAMPAL 5-HT AND 5-HIAA LEVELS IN VTG LESION AND NON-LESION ANIMALS

Treatment	n	Hippocampus 5-HT (ng/g)	Hippocampus 5-HIAA (ng/g)	Striatum 5-HT (ng/g)	Striatum 5-HIAA (ng/g)
Sham-Operated Control	8	416 ± 27	743 ± 55	607 ± 22	937 ± 34
MR-Cannula Alone	8	370 ± 33	564 ± 30*	600 ± 22	898 ± 37
VTG Lesion + MR-Cannula	6	179 ± 31*†	343 ± 28*†	619 ± 26	767 ± 35

*Significantly different from sham-operated controls ($p<0.01$).

‡Significantly different from MR-cannula alone ($p<0.01$, Newman-Keuls' multiple range test).

Effects of VTG lesions on regional 5-HT and 5-HIAA levels. Hippocampal and striatal 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Mean±S.E.M. ng/g wet tissue mass) in VTG lesion and control animals. Data entries represent Mean±S.E.M.

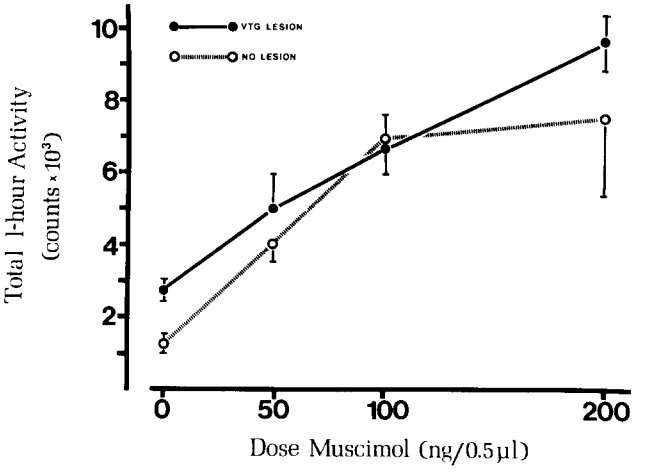

FIG. 8. Effects of VTG lesions on muscimol-induced hyperactivity Dose-response relationship for muscimol injections through cannulae chronically implanted in the median raphe nucleus of rats with bilateral lesions in the ventral tegmental nuclei of Gudden (VTG, n=6) or control operations (n=8). Total activity scores (Mean± S.E.M.) for the one hour period following muscimol injection.

in Experiment 2, again with activity counts recorded every 15 minutes for one hour post-injection. At the end of the experiment, the animals were sacrificed by decapitation, their hippocampal and striatal 5-HT levels assayed by HPLC, and their brainstems fixed in buffered formalin for histological verification of cannula and mesencephalic 5,7-DHT injection sites.

Results

Histological Analysis. Of the animals which survived the entire experiment, 7 intraventricular 5,7-DHT, 7 intraventricular control, 7 mesencephalic 5,7-DHT, and 6 mesencephalic control subjects had cannulae well localized within the B-8 5-HT cell group, dorsal to the interpeduncular nucleus. In addition, the sites of the intramesencephalic 5,7-DHT and vehicle injections were examined and all found to be appropriately localized within the course of the ascending 5-HT projections through the ventral tegmental area of Tsai, or immediately dorsal to the rostral tip of the interpeduncular nucleus (Fig. 9). The histological appearance of the 5,7-DHT and vehicle injection sites were virtually indistinguishable.

Biochemical analysis. The 5-HT and 5-HIAA concentrations in the neostriata and hippocampi of the intracerebroventricular vehicle injected control animals (Table 2) were virtually the same as those for the sham-operated control animals in Experiment 4 (Table 1). Intraventricular 5,7-DHT reduced the 5-HT concentrations in both the striatum (95%) and hippocampus (83%) as compared to intraventricular vehicle injections. The intramesencephalic 5,7-DHT injections also significantly reduced the 5-HT contents of the striatum (77%) and the hippocampus (59%) as compared to the intra-midbrain vehicle injections. Similar reductions were seen in the regional concentrations of the 5-HT metabolite, 5-HIAA. Intramesencephalic vehicle injections in comparison to the intraventricular vehicle injections, produced a small (22%), insignificant reduction in the hippocampal 5-HT concentration, but a significant (37%) fall in hippocampal 5-HIAA content (Table 2). This observation, supported by the histological data, suggests that the intra-midbrain vehicle injections interrupted some of the 5-HT fibers which innervate the hippocampus.

Behavioral analysis. An ANOVA (three factor mixed design—repeated measures on one factor) [6] of the pre-drug baseline activity levels of the 4 groups of rats failed to reveal any significant 5,7-DHT lesion effect, regardless of the mode of its production (intraventricular versus intramesencephalic). However, an ANOVA (three factor mixed design—repeated measures on two factors) [6] of the effects of intra-raphe muscimol injections showed the following. The effects of the dose of muscimol were significant, $F(3,72)=6.28$, $p<0.002$, as were the effects of time after injection, $F(3,72)=14.59$, $p<0.0001$. In addition, the effects of lesion group and of

TABLE 2

STRIATAL AND HIPPOCAMPAL 5-HT AND 5-HIAA LEVELS IN ANIMALS WITH INTRAVENTRICULAR OR INTRA-MIDBRAIN 5,7-DHT LESIONS

Treatment	n	Hippocampus 5-HT (ng/g)	Hippocampus 5-HIAA (ng/g)	Striatum 5-HT (ng/g)	Striatum 5-HIAA (ng/g)
Intraventricular Vehicle	7	464 ± 49	741 ± 64	526 ± 61	824 ± 76
Intraventricular 5,7-DHT	7	76 ± 13*†‡	60 ± 18*‡	28 ± 6*†‡	102 ± 23*†‡
Intra-Midbrain Vehicle	6	362 ± 34	470 ± 62*	542 ± 99	757 ± 69
Intra-Midbrain 5,7-DHT	7	148 ± 15*‡	168 ± 41*‡	123 ± 35*‡	327 ± 54*‡

*Significantly different from intraventricular vehicle ($p<0.01$);
†Significantly different from intra-midbrain 5,7-DHT ($p<0.01$);
§Significantly different from intra-midbrain vehicle ($p<0.01$, Newman-Keuls' multiple range test).

Effect of 5,7-DHT on regional 5-HT and 5-HIAA levels. Hippocampal and striatal 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Mean±S.E.M. ng/g wet tissue mass) in control animals and animals with intra-midbrain and intraventricular 5,7-dihydroxytryptamine (5,7-DHT) lesions.

muscimol dose interacted, $F(9,72)=7.26$, $p<0.001$. Individual comparisons of the total 60 minute post-injection activity level for each group confirmed that both types of forebrain 5-HT-reducing lesion markedly attenuated the effects of intra-raphe muscimol (Fig. 10).

Conclusion

The data obtained from the present experiment suggest that the hyperkinetic effect of intra-midbrain raphe injections of muscimol depend on an intact ascending 5-HT fiber system.

GENERAL DISCUSSION

Overall, the present series of experiments indicates that the injection of the GABA agonist, muscimol, into the mesencephalic raphe results in a dose-dependent hyperkinetic response which is mediated by an ascending serotonergic system. The present observations also suggest that GABA receptors within the midbrain raphe regulate the discharge rate of 5-HT neurons whose forebrain efferents modulate motor systems, and that the benzodiazepines can modify the activity of these systems centrally.

In the first experiment we replicated the previous findings [44] that acute microinjection of muscimol (100 ng) into the dorsal raphe nucleus produces hyperactivity as measured in photocell chambers. In addition, we found that the median raphe nucleus was even more sensitive to muscimol (100 ng) than the dorsal raphe nucleus, the magnitude of the hyperkinetic effect being four times greater after injection into the former site. The acute injection paradigm employed in the earlier study [44], and in Experiment 1, required that the subjects be tested immediately upon awakening from the ether anesthetic. Thus, it was not possible to discern whether the hyperactivity produced was due to muscimol alone, or to the interaction between muscimol, ether and the trauma of surgery.

In order to circumvent this problem, we performed a muscimol dose response-analysis using animals with cannulae implanted chronically into either the dorsal or the median raphe nucleus (Fig 3). Since each animal was to receive each drug dose, this experiment was carried out using a Latin square design in order to determine any order of treatment effects. We found that injection of muscimol into the median raphe nucleus produced a significant elevation in activity level at a dose (50 ng) one-fourth that required to produce a similar effect when injected into the dorsal raphe nucleus. The magnitude of the hyperkinetic response following the injection of an optimal dose of muscimol (100 ng) into the median raphe nucleus, moreover, was at least 1.5 times that after such an injection (200 ng) into the dorsal raphe nucleus (Fig. 4). Importantly, there was no effect of order of treatment. Therefore, subsequent experiments could be performed using a randomized block design.

The observation that the median raphe nucleus is four times more sensitive to muscimol than the dorsal raphe nucleus raises the possibility that the hyperkinesis following muscimol injections into the dorsal raphe nucleus may be due, at least in part, to a spread of the drug to the more sensitive median raphe site. We have begun a series of in vivo autoradiographic studies to determine the time course and the extent of the spread of radiolabel following injections of various doses of ^{3}H-muscimol through cannulae chronically implanted in the dorsal and median raphe nuclei of rats. Our preliminary results suggest that following injections of ^{3}H-muscimol (50 ng) into the median raphe nucleus, the greatest density of label remains concentrated around the guide cannula tip, even 60 minutes post-injection. A light, but significant density of label by this time, however, has spread nearly 1.0 mm ventrally, caudally and rostrally. Such a spread of muscimol from the tip of a cannula in the dorsal raphe nucleus would reach the median raphe nucleus. It is of interest to note in this regard that in the previous studies [44] decreases in 5-HT and 5-HIAA concentrations were found in

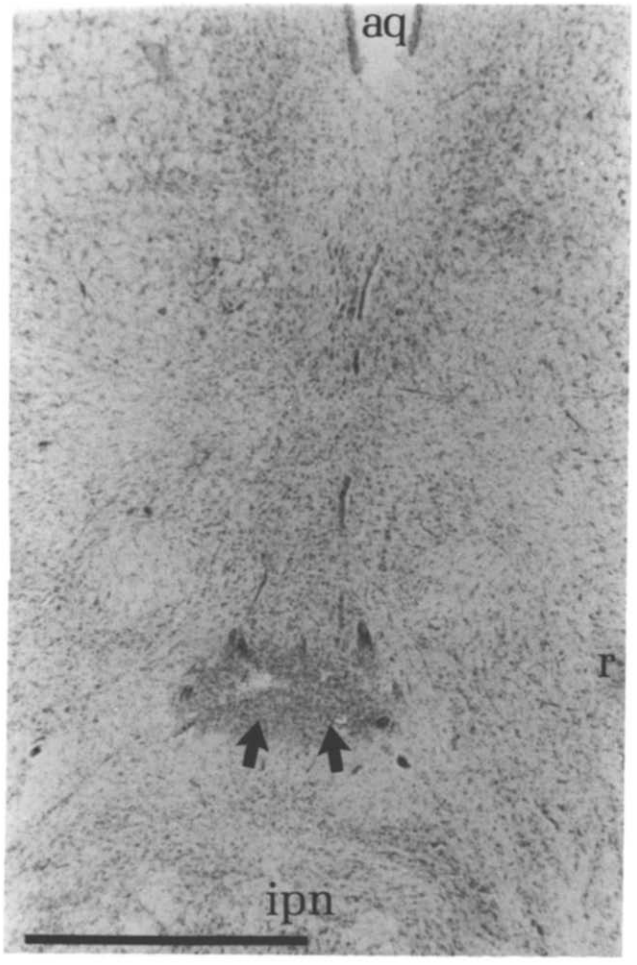

FIG. 9. Photomicrograph of a midbrain 5,7-DHT injection site. Site of a typical intra-mesencephalic 5,7-dihydroxytryptamine (5,7-DHT) injection (4.0 μg in 2.0 μl vehicle, bilaterally) is demarcated by a glial scar (arrows). Cresylecht violet stained 50 μm coronal section. Bar represents 1 mm. Abbreviations: aq=cerebral aqueduct of Sylvius, ipn=interpenduncular nucleus, r=red nucleus.

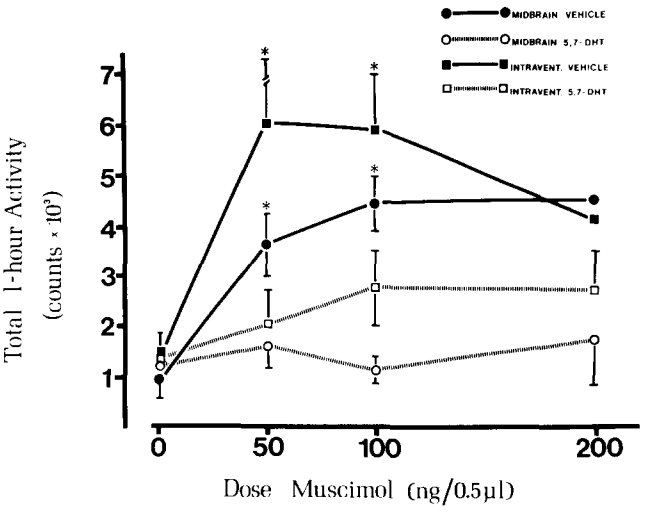

FIG. 10. Forebrain 5-HT depletion blocks the effect of muscimol. Dose response relationship for the injection of muscimol (0, 50, 100 or 200 ng) through cannulae chronically implanted in the median raphe nucleus of rats which had received 5,7-dihydroxytryptamine (5,7-DHT) injections into the cerebral ventricles (75 μg bilaterally), or into the ascending 5-HT fibers at the level of the red nucleus (4.0 μg bilaterally). Group mean (±S.E.M.) activity scores for the 1 hour post-injection period are presented. *Significantly elevated over saline-injected control condition ($p<0.01$, Newman-Keuls' test for related means).

the hypothalamus, but not in the neostriatum, following the injection of muscimol (25–100 ng) into the dorsal raphe nucleus. As stated in the introduction, substantial evidence has been advanced demonstrating that 5-HT fibers afferent to the neostriatum arise predominantly in the dorsal raphe nucleus (B-7 5-HT cell group), whereas the 5-HT projection to the hippocampus originates principally in the B-8 5-HT cell group, which overlaps the caudal linear and median raphe nuclei [51]. In addition, it has been reported [15] that muscimol injections into the median raphe nucleus reduce 5-HT turnover in the hippocampus. Thus, the observed hyperkinetic response to intra-dorsal raphe muscimol injections [44] might have been due to a spread of the muscimol to the median raphe nucleus (B-8 5-HT cell group). This hypothesis currently is under investigation in our laboratory.

As stated earlier, there is evidence to implicate GABA as a neurotransmitter in both the dorsal [3, 16, 17, 32, 38] and the median raphe nucleus [15]. Many of the behavioral and physiological effects of the benzodiazepines are thought to be due to their facilitation of GABA-ergic transmission [8, 9, 11, 16, 38]. If the effects of intra-mesencephalic administration of muscimol are due to activation of GABA receptors in the raphe nuclei, they should be potentiated by benzodiazepines (for example, chlordiazepoxide) and blocked by the GABA-receptor antagonist, bicuculline.

In a preliminary study [45], we found that intraperitoneal administration of chlordiazepoxide in doses greater than 3.8 mg/kg produced decreases in locomotor activity as measured in dark photocell chambers to which the animals had been habituated previously. Lower doses produced no effect on activity level. These findings are consistent with those obtained by other laboratories [7, 24, 31, 41]. These investigators have reported that whereas the benzodiazepines enhance exploratory behavior in novel environments, they have little effect on activity level when the animals are tested in settings to which they have been habituated.

We found (Experiment 3) that peripheral administration of chlordiazepoxide, in the sub-ataxic dose of 3.8 mg/kg, did not itself affect activity level, but enhanced the locomotor activity response to low doses (25 and 50 ng) of muscimol injected into the median raphe nucleus. Conversely, a subconvulsant dose of bicuculline (1.1 mg/kg) completely blocked the response to 50 and 100 ng of muscimol.

The ventral tegmental nuclei of Gudden (VTG) lie just dorsolateral to the median raphe nucleus. Bilateral electrolytic lesions in the VTG produce hyperactivity similar to that seen after electrolytic lesions in the median raphe nucleus. The VTG are sites of dense benzodiazepine receptor localizations [57,58]. In vitro autoradiographic studies currently in progress in our laboratory confirm this finding [46]. Furthermore, benzodiazepine receptors usually are associ-

ated with GABA receptors [9, 11, 37, 38]. Although not a particularly rich source of GABA or GAD [32], the VTG receive a significant afferent projection from the ipsi- and contra-lateral dorsal tegmental nuclei of Gudden [5,40], which contain high levels of GAD. It seemed entirely possible, therefore, that the hyperactivity induced by intraraphe muscimol injections could be due to diffusion of the drug to GABA-ceptive sites located in the VTG. We, therefore, destroyed the VTG in order to determine whether this manipulation would attenuate the hyperkinetic effect of intra-raphe muscimol injections. We found, in agreement with earlier findings [29], that the VTG lesions increased open field activity and facilitated the acquisition of a two-way conditioned avoidance response. In the darkened activity chambers, the VTG lesion animals also manifested higher baseline levels of activity than controls, but the lesions failed to attenuate the facilitatory effects of muscimol. Thus, it appears that the effects of intra-raphe muscimol on locomotor activity and of VTG lesions on this behavior, are independent phenomena.

Subsequently, forebrain 5-HT was depleted by injecting the specific serotonin neurotoxin, 5,7-dihydroxytryptamine, into either the lateral cerebral ventricles or into the midbrain tegmentum. Injection of 5,7-dihydroxytryptamine (10 μg base) directly into the dorsal and median raphe nucleus, even in the absence of biogenic amine uptake inhibitors, fails to affect either spinal and forebrain norepinephrine (NE) concentrations, or striatal dopamine levels [28]. It has been reported, furthermore, that pretreatment of animals with secondary amine tricyclic antidepressants, such as desmethylimipramine, prevents the uptake of and subsequent destruction of NE neuronal elements by 5,7-dihydroxytryptamine [4,18]. 5,7-Dihydroxytryptamine lesions markedly attenuated to the locomotor response produced by muscimol injections into the median raphe nucleus. These data suggest that midbrain GABA neurons modulate activity level in the rat through a direct action on mesencephalic 5-HT neurons.

As a result, we hypothesize that when muscimol is injected directly into the midbrain raphe nuclei, it suppresses the firing rate of serotonergic neurons by activating local GABA receptors. This, in turn, elicits locomotor hyperactivity by releasing hippocampal [55] or substantia nigra (see below) neurons from a tonic serotonergic inhibitory influence. Intraventricular and intramesencephalic 5,7-dihydroxytryptamine lesions, however, did not alter baseline activity level in our photocell chambers. Biochemical analysis showed that these lesions resulted in a degeneration of forebrain 5-HT efferents, but histological analysis indicated that their perikarya of origin were spared, possibly because of sustaining collaterals. It may be that in order to induce hyperactivity the 5-HT cell bodies associated with the midbrain raphe nuclei themselves must be destroyed. Also, during the 2 week post-lesion recovery period, the motor systems of the animals may adapt to the loss of the ascending 5-HT projections allows a new, compensated "steady state" to develop.

The neuronal mechanisms by which a GABA-ergic inhibition of raphe 5-HT cells might produce elevations in locomotor activity is largely a matter of speculation. There is a substantial amount of evidence to support the existence of inhibitory 5-HT projections from the dorsal and median raphe nuclei to the substantia nigra in the rat [13, 14, 36, 54]. It has been reported that 5,7-dihydroxytryptamine injections into the median raphe nucleus, if placed 0.3 mm lateral to the midline, produce a "unilateral" lesion [19]. Such lesions cause rats to rotate in a direction contralateral to the site of injection, the rotation being blocked by haloperidol. The authors also found a significant correlation between the rate of rotation, the decrease in cortical 5-HT turnover, and the increase in striatal dopamine turnover. Moreover, they found that injections of 5,7-dihydroxytryptamine into the substantia nigra itself produced biochemical and behavioral changes similar to those following 5,7-dihydroxytryptamine injections into the median raphe nucleus.

The nigrostriatal dopamine pathway is an important component of the extrapyramidal motor system. The indirect dopamine agonist, amphetamine, and the dopamine-receptor agonist, apomorphine, both can induce turning in rats with unilateral lesions in the nigrostriatal dopamine system [52,53]. These drugs, however, induce turning in opposite directions. Amphetamine is thought to produce ipsilateral turning by releasing dopamine from intact contralateral striatal nerve terminals, while apomorphine is thought to induce contralateral turning by stimulation of supersensitive dopamine receptors in the denervated ipsilateral striatum.

If unilateral stimulation of striatal dopamine receptors produces rotation in a direction contralateral to the site of stimulation, then bilateral stimulation of striatal dopamine receptors might produce a non-rotational hyperactivity. In our experiments, midline intra-raphe injections of muscimol hypothetically would suppress the inhibitory raphe-substantia nigra 5-HT pathway, producing a bilateral increase in the firing rates of nigral dopamine neurons. This, in turn, would lead to a non-rotational, stereotypic hyperkinesis.

In summary, the present results suggest that midbrain raphe 5-HT neurons exert a tonic inhibitory influence on CNS systems which coordinate locomotor behavior. Temporary removal of this inhibition by administering a GABA agonist, such as muscimol, into the mesencephalic raphe results in hyperactivity. Thus, this forebrain 5-HT system appears to be regulated by raphe GABA interneurons, whose post-synaptic effects can be facilitated following the administration of benzodiazepine drugs.

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