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Evidence Against Serotonin Involvement in the Hyperactivity Produced by Injections of Muscimol Into the Median Raphe Nucleus[1]

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WIRTSHAFTER, D., M. A. KLITENICK AND K. E. ASIN. *Evidence against serotonin involvement in the hyperactivity produced by injections of muscimol into the median raphe nucleus.* PHARMACOL BIOCHEM BEHAV 27(1) 45–52, 1987.— Microinjections of muscimol into the median raphe nucleus were found to result in pronounced hyperactivity which could not be attenuated by the serotonin depletion produced either by systemic treatment with p-chlorophenylalanine or by intra-raphe injections of 5,7-dihydroxytryptamine. Furthermore, hyperactivity could not be produced by intra-median raphe injections of serotonin or of fenfluramine, compounds which would be expected to inhibit serotonergic raphe cells. These results argue strongly against an essential involvement of serotonin in mediating the effects of intra-median raphe muscimol injections. Muscimol failed to produce hyperactivity, however, when injected into rats who had previously received an electrolytic median raphe lesion. This finding suggests that muscimol injected into the median raphe produces hyperactivity as a result of an action on local cell bodies, rather than by diffusion to a distant site. The simplest explanation of the current results is that muscimol injected into the median raphe produces hyperactivity as a result of an inhibition of nonserotonergic cells within the median raphe nucleus.

Median raphe nucleus GABA Muscimol Serotonin Hyperactivity

CONSIDERABLE evidence suggests that the median raphe nucleus (MR) may play an important role in the control of locomotor activity. For example, many authors have found that electrolytic MR lesions lead to a dramatic increase in locomotor activity in a variety of situations [4, 5, 14, 18, 23, 25, 35, 37]. Although the effect is not as large as that seen after electrolytic lesions, hyperactivity can also be produced by intra-MR injections of the axon sparing excitotoxic agent ibotenic acid [4], demonstrating that cells within the vicinity of the MR must play a role in the control of locomotion. This conclusion is further supported by the report of Sainati and Lorens [31,32] that acute injections of the GABA-A agonist muscimol into the MR of conscious rats leads to a dramatic increase in activity.

It is well known that the MR is one of the major sources of ascending serotonergic projections to the forebrain. The question naturally arises, therefore, as to the role played by these serotonergic cells in the control of locomotor activity. Many studies have now demonstrated that specific damage to ascending serotonergic systems produced by injections of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the MR, the ascending serotonin bundles, or the lateral ventricles, fails to produce hyperactivity of the type seen after electrolytic or ibotenic lesions of the MR [4, 13, 15, 16, 25]. Similar results have been obtained following pharmacological manipulation of the serotonergic system through systemic administration of the serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) [10,22] or the serotonin neurotoxin p-chloroamphetamine (PCA) [27,34]. All of these findings suggest that serotonin depletion cannot by itself account for the enhanced locomotor activity seen after electrolytic or ibotenic acid lesions of the MR, and point to the possible involvement of nonserotonergic cells in these effects. In this regard, it has been estimated that as many as two-thirds of the perikarya in the MR are nonserotonergic [6, 9, 24, 28].

In contrast to the above findings, Sainati and Lorens have provided evidence that the hyperactivity seen after intra-MR injections of muscimol results from inhibition of serotonin containing cells [31]. These authors reported that serotonin depleting injections of 5,7-DHT into the lateral ventricles or the ventral tegmental area failed to influence spontaneous locomotor activity but severely attenuated the hyperactivity produced by injections of muscimol. In order to account for

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the fact that the 5,7-DHT lesions did not produce hyperactivity by themselves, the authors postulated that only an acute reduction of serotonergic activity is able to increase locomotion and that the effects of chronic depletions might be offset by the development of various compensatory mechanisms. If correct, this interpretation would obviously have important implications for our understanding of the functions of central serotonergic systems in behavior.

Although this theory is internally consistent, certain considerations suggest the existence of some difficulties with it. For example, if the engagement of compensatory mechanisms, such as sprouting or deinnervation supersensitivity, were able to completely eliminate the effects that chronic serotonin depletion would otherwise have on locomotor activity, these mechanisms must work with extreme rapidity, since initial levels of locomotor activity are normal 24 hours after injections of 5,7-DHT [12]. Also, studies using ibotenic acid lesions have demonstrated that destruction of nonserotonergic cells within the paramedian tegmentum can lead to long lasting increases in locomotion [1]. In order to account for these findings, the theory that muscimol-induced hyperactivity is entirely due to an inhibition of serotonergic neurons would require the postulation of two independent sets of cells modulating locomotion within the vicinity of the MR. One of these (the serotonin cells) would have to be sensitive to muscimol but produce hyperactivity only if acutely inhibited, while the other (the nonserotonergic cells) would have to be insensitive to muscimol but be capable of mediating chronic changes in locomotor activity. Although this arrangement is by no means impossible, it does demonstrate that the postulation of intricate mechanisms is necessary to reconcile other data with the notion that inhibition of serotonergic cells completely underlies the effects of intra-MR muscimol on activity.

In view of the above considerations, the current series of experiments was designed to reexamine the involvement of serotonergic mechanisms in the hyperactivity induced by injections of muscimol into the MR. The results of these studies strongly suggest that serotonergic mechanisms do not play a critical role in mediating this effect of muscimol. A preliminary report of some of the current results has been published in abstract form [21].

EXPERIMENT 1

This experiment examined the effects of PCPA-induced depletions of serotonin on muscimol-induced hyperactivity. If the muscimol effect on activity is mediated by an acute reduction in the activity of serotonergic cells, one would expect that it would be antagonized by prior depletion of serotonin, as was reported by Sainati and Lorens [31] for the case of 5,7-DHT lesions.

METHOD

Subjects

Subjects were 21 male Sprague-Dawley derived rats, weighing 300±20 g at the time of surgery, obtained from a colony maintained by the University of Illinois at Chicago. Rats were randomly divided into 3 treatment groups: P×1, n=7; P×2, n=8; and V×2, n=6 and were housed individually in wire-mesh cages and kept in a temperature and light controlled (12:12 hour light:dark cycle) environment. Food and water were available ad lib.

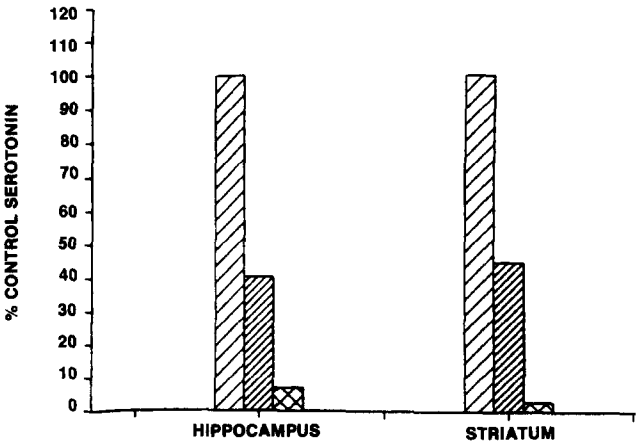

FIG. 1. Serotonin levels in the hippocampus and striatum for subjects receiving vehicle injections or one or two injections of PCPA (300 mg/kg). Wide striped column: vehicle, narrow striped column: PCPA ×1, hatched column: PCPA ×2.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg, IP). Using standard stereotaxic techniques, a stainless steel 21 gauge guide cannula was implanted aimed at coordinates AP: −0.3; H: −2.1; L: 0.0 [29]. The guide cannula was lowered on the midline following retraction of the superior sagittal sinus [36] and was anchored to the skull with dental cement. An obdurator made of 27 gauge stainless steel wire was inserted into the guide cannula so that it extended an additional 2 mm into the brain. Sodium sulfathiazol (Merck & Co., Rahway, NJ) was applied topically to the wound followed by a prophylactic injection of 0.5 cc Flo-Cillin (SC, Bristol Laboratories, Syracuse, NY).

Apparatus

Locomotor activity was measured in an infrared photocell cage 71.5 cm by 71.5 cm by 27 cm. A 9 by 9 array of holes 3.5 cm in diameter perforated the floor. Four infrared beams were positioned 3.5 cm above the floor in a 2 by 2 grid. The walls and floor were painted black and a clear Plexiglas lid covered the cage. Beam interruptions were registered in an adjoining room every 4 min on a counter controlled by BRS digital logic.

Intra-MR Injection

All microinjections into the MR were made following a one hour habituation period. A 27 gauge injection cannula connected to a Hamilton microsyringe by polyethylene tubing was inserted into the guide cannula so that the injection tip extended 2 mm beyond the guide. Intra-MR injections were made in a volume of 0.5 μl over 1 min; the injection cannula was left in place for an additional 30 sec to allow for diffusion away from the tip. Subjects were handheld for the duration of the injection after which the obdurator was replaced and the subjects were returned to the activity cage.

Procedure

All animals were handled daily during the 7 days of postoperative recovery. On post-op day 8 each rat was individually placed in the photocell cage and was allowed to

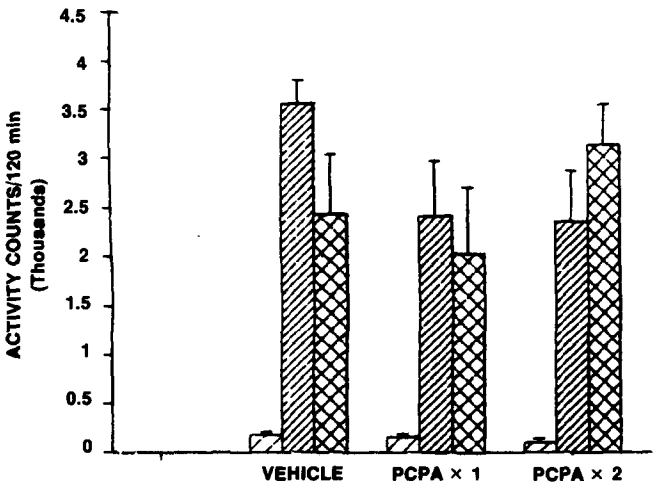

FIG. 2. Locomotor activity following intra-MR injections of saline or muscimol. Subjects received muscimol both before (musc. pre) and after (musc. post) systemic injections of PCPA or its vehicle. Wide striped column: saline, narrow striped column: musc. pre, hatched column: musc. post.

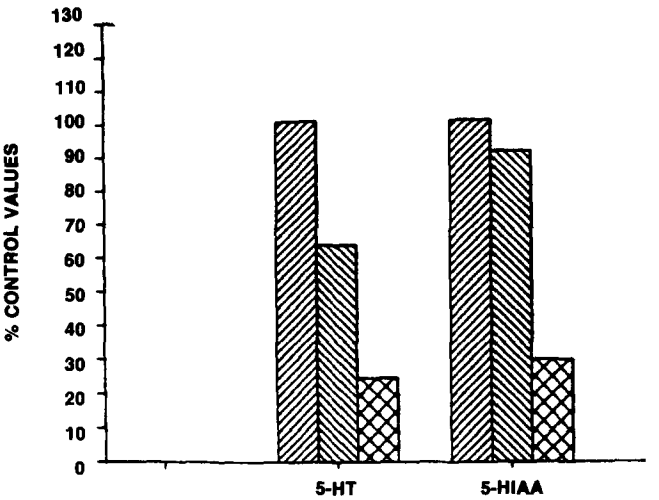

FIG. 3. Hippocampal levels of serotonin and 5-HIAA for unoperated control animals and for subjects receiving intra-MR injections of 5,7-DHT or its vehicle. Upward striped column: unop. control, downward striped column: vehicle, hatched column: 5,7-DHT.

habituate to it for 1 hour. After being gently removed from the photocell cage, each rat was then given an intra-MR injection of 0.5 μl of isotonic saline and was returned to the photocell cage for an additional 2 hours. A subsequent photocell cage session was conducted three days later (post-op day 11) following an intra-MR injection of 100 ng muscimol in 0.5 μl of isotonic saline, an optimal dose according to Sainati and Lorens [31]. Twenty-four hours after the muscimol run (post-op day 12) groups P×1 and P×2 were treated with PCPA (300 mg/kg, IP), group V×2 received the vehicle (3 ml/kg). PCPA was suspended in 2% Tween-80 at a concentration of 100 mg/ml. PCPA and vehicle treatments were repeated 2 days later for groups P×2 and V×2, respectively. A final test of activity was then conducted on post-op day 16 in which subjects were given an intra-MR injection of muscimol (100 ng).

Biochemistry and Histology

Owing to the transient nature of the biochemical effects of PCPA, it was necessary to examine serotonin depletion in subjects other than those used in the behavioral studies. Eighteen additional subjects thus were given PCPA or vehicle treatments equivalent to those described above. Six unoperated subjects each received either a single PCPA injection four days before sacrifice, two PCPA injections two and four days before sacrifice, or vehicle injections two and four days before sacrifice. Rats were killed by cervical fracture and the brain was quickly removed and the hippocampus and striatum dissected out on ice. The tissue samples were weighed, homogenized in acidified butanol and fluorometrically assayed for serotonin (5-HT) content by the method of Jacobowitz and Richardson [17].

Subjects used in the behavioral studies were perfused transcardially, under deep pentobarbital anesthesia, with saline followed by 10% formalin. The brains were then removed and stored in formalin for at least one week after which time 64 μ cryostat sections were taken through the extent of the cannula tract and stained with cresyl violet.

RESULTS

The results of the fluorometric assay are shown in Fig. 1. Analysis of these data by a one-way analysis of variance (ANOVA) indicated that PCPA treatment produced a significant depletion of both hippocampal and striatal serotonin relative to control animals, $F(2,15)=12.46$, $p<0.01$. The magnitude of this effect was dependent on the number of injections.

Histological examination showed that all cannulae terminated with the MR, most placements being at the level of the ventral tegmental nuclei of Gudden about 2.5 mm dorsal to the nucleus reticularis tegmenti pontis (NRTP).

Behavioral results are shown in Fig. 2 where it can be seen that PCPA pretreatment did not attenuate the locomotor response to intra-MR microinjections of muscimol. Two hour total activity scores following muscimol injections were analyzed by a 3×2 (PCPA dose × pre/post-PCPA treatment) ANOVA with repeated measures on the second factor. Neither the main effects nor the interaction approached significance ($p>0.3$) indicating that PCPA treated animals did not show a reduced muscimol response with respect either to their own pre-PCPA baselines or with respect to vehicle treated control animals. Relative to their saline baselines, all groups showed a highly significant hyperactivity in response to both muscimol injections ($p<0.01$).

DISCUSSION

The results of Experiment 1 demonstrate that injections of PCPA produced large depletions of serotonin, the magnitude of the effect being dependent on the number of injections. None the less, treatments with PCPA which depleted serotonin by more than 90% failed to produce any antagonism of the locomotor response to intra-MR injections of muscimol. The magnitude of the serotonin depletion produced here was larger than that reported by Sainati and Lorens [31] to be effective in antagonizing the muscimol effect. Clearly, these results are not supportive of the notion

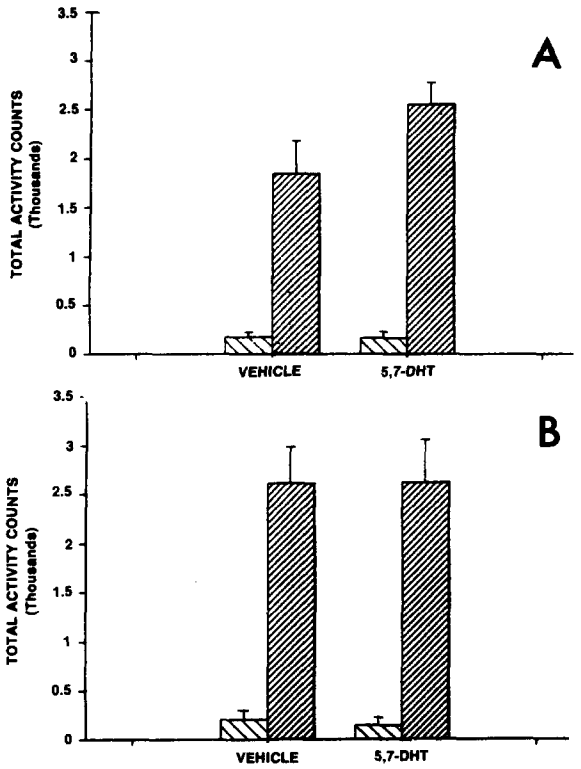

FIG. 4. Locomotor activity following intra-MR injections of saline or muscimol before (A) and after (B) intracranial injections of 5,7-DHT or its vehicle. Wide striped column: saline, narrow striped column: muscimol.

that muscimol-induced hyperactivity results from an acute inhibition of serotonergic activity. Results with PCPA similar to those reported here have recently been obtained by Lorens (personal communication).

EXPERIMENT 2

Experiment 2 was designed to extend the results of the first experiment by examining the effects of 5,7-DHT-induced serotonin depletions on the hyperactivity produced by intra-MR injections of muscimol. The 5,7-DHT was injected into the MR through the chronically implanted guide cannula. This technique allowed for testing of individual subjects before and after 5,7-DHT treatment.

METHOD

Subjects and Surgery

Subjects were similar to those described in the first experiment. Fourteen rats were prepared with chronic indwelling guide cannula, as described in Experiment 1 and were randomly divided into 5,7-DHT (n=8) and vehicle (n=6) groups.

Procedure

Locomotor activity was measured, as described in Experiment 1, after an intra-MR injection of saline and again three days later following an intra-MR injection of muscimol. Twenty-four hours later (post-op day 12) each rat was injected with desipramine hydrochloride (DMI, 25 mg/kg, IP) 45 min prior to 5,7-DHT treatment. Under light ether anesthesia 7.5 μg of 5,7-dihydroxytryptamine creatinine sulfate (calculated as the salt) in 1.5 μl of isotonic saline or the saline vehicle was injected into the MR over 8 min; the injection cannula was left in place an additional 3 min. Animals were allowed to recover for 12 days before behavioral testing was resumed. Activity was then measured in each rat after saline and 3 days later following muscimol.

Biochemistry and Histology

Seven randomly selected 5,7-DHT treated rats, four vehicle injected animals and 10 unoperated control animals were studied biochemically. Rats were killed by cervical fracture and the brains were quickly removed and placed on a cold glass plate over ice. The hippocampus was dissected out, weighed, homogenized in mobile phase [20], centrifuged and the supernatant kept at −80°C until it was assayed for serotonin (5HT), and 5-hydroxyindoleacetic acid (5-HIAA) content using reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection according to the method of Kilts, Breese and Mailman [20]. 5-Hydroxy-n-methyltryptamine oxalate was used as the internal standard. The pH of the mobile phase was adjusted to 4.2 and the column maintained at a temperature of 30°C. The remaining brainstem was stored in a 10% solution of formalin for at least one week. Animals not assayed were perfused as described in Experiment 1. Frozen 64 μm sections were then cut through the region of the MR and were stained with cresyl violet. Brains were examined microscopically to verify cannula placement and to assess any nonspecific damage caused by injection of the neurotoxin.

RESULTS

As in the first experiment, histological examination revealed that all cannulae terminated within the MR. Little or no evidence of gross nonspecific damage was present in the 5,7-DHT treated group. Biochemical results are shown in Fig. 3 where it can be seen that, relative to intact control animals, injections of 5,7-DHT into the MR resulted in a much larger depletion of hippocampal serotonin and 5-HIAA than that seen after vehicle injections. Analysis of this data by means of one way ANOVA's indicated a highly significant effect of treatments on levels of both serotonin, $F(2,18)=56.52$, $p<0.001$, and 5-HIAA, $F(2,18)=10.19$, $p<0.001$. Post hoc comparisons indicated that, for both biochemical measures, 5,7-DHT injections produced significantly larger depletions than did vehicle injections ($p<0.02$).

Total activity scores following intra-MR injection of saline and muscimol before and after 5,7-DHT are shown in Fig. 4. These data were analyzed by a 2×2×2 (5,7-DHT/vehicle × pre/post-5,7-DHT or vehicle × saline/muscimol) ANOVA with repeated measures on the last two factors. The analysis indicated a highly significant effect of muscimol treatment, $F(1,12)=139.89$, $p<0.0001$, but the other main effects and interactions all failed to approach statistical significance ($F<1$). This result indicates that 5,7-DHT treated rats did not show alterations in their locomotor response to either muscimol or saline with respect to either their own pre-5,7-DHT baselines, or with respect to vehicle injected control animals. A similar analysis conducted on locomotor activity during the one hour pre-injection habitu-

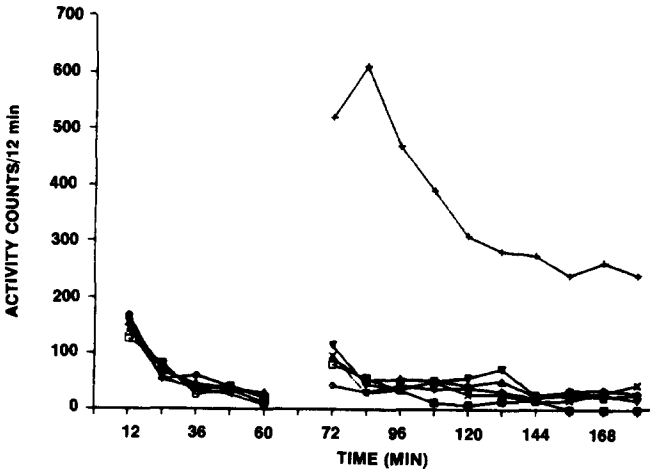

FIG. 5. Locomotor activity across time following intra-MR injections of saline (□), 100 ng muscimol (+), 10 μg serotonin (◇), 20 μg serotonin (△), 10 μg fenfluramine (×) or 20 μg fenfluramine (▽).

ation periods failed to demonstrate any effect of the 5,7-DHT treatments on spontaneous locomotor activity (data not shown). Furthermore, there was no tendency for the extent of serotonin depletion to correlate with the magnitude of the behavioral response to muscimol injections (r=−.291).

DISCUSSION

The results of Experiment 2 demonstrate that the locomotor response to intra-MR injections of muscimol is not altered by prior serotonin depleting injections of 5,7-DHT into the MR. Relative to vehicle injected subjects, the magnitude of the 5-HT depletion produced by our 5,7-DHT injections is comparable to that observed by Sainati and Lorens [31] after injections into the ventral tegmental area which markedly reduced the magnitude of the muscimol effect on locomotor activity. Furthermore, considering individual subjects, there was no tendency for the magnitude of serotonin depletion to correlate with alterations in the muscimol response. These results are in agreement with those of the first experiment and further support the suggestion that serotonergic systems arising from the MR do not, in fact, play an essential role in the locomotor response to intra-MR injections of muscimol.

EXPERIMENT 3

Electrophysiological studies have indicated that serotonergic cells are extremely sensitive to inhibition by a variety of serotonin agonists [1, 2, 33], an effect which is presumably mediated by cell body or dendritic autoreceptors. It is interesting that serotonergic cells within the raphe nuclei seem to be more sensitive to the inhibitory effects of these compounds than do nonserotonergic raphe cells [1,2]. If acute inhibition of serotonergic cell firing results in hyperactivity, as suggested by Sainati and Lorens [31], intra-MR injections of serotonin autoreceptor agonists should, like muscimol, increase locomotion. This possibility was examined in the current study by comparing the effects of intra-MR injections of muscimol, serotonin and fenfluramine. Both serotonin and fenfluramine have been shown in iontophoretic studies to be potent inhibitors of serotonin cell activity [1,2].

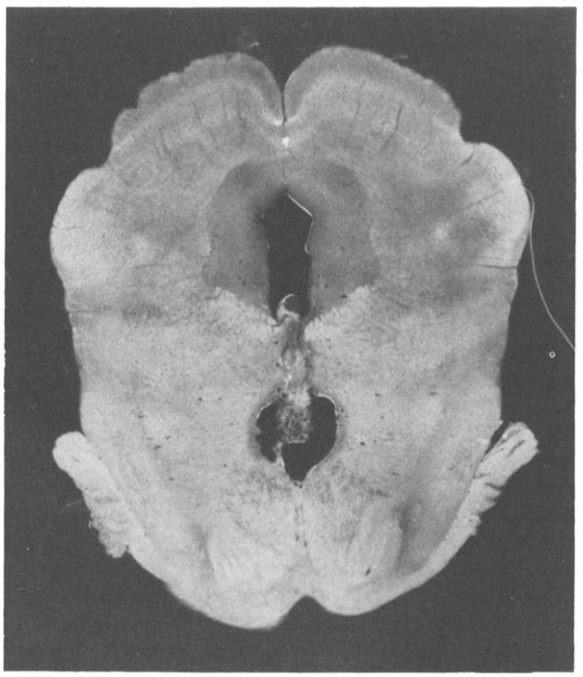

FIG. 6. Photograph of a typical 16 sec electrolytic MR lesion showing the cannula tract terminating within the lesion cavity.

METHOD

Five rats, similar to those used in the first two experiments, were prepared with chronic indwelling guide cannula and allowed to recover for 7 days as described above. Histological procedures were as described in Experiment 1.

Drugs

All drugs were dissolved in isotonic saline. Serotonin bitartrate (Sigma) in doses of 10 μg/0.5 μl and 20 μg/1.0 μl (calculated as the salt) and fenfluramine (Sigma) 10 and 20 μg/0.5 μl were used for intra-MR injections in addition to muscimol (100 ng/0.5 μl) and isotonic saline (0.5 μl).

Procedure

Locomotor activity was measured, as described in Experiment 1, at 3 day intervals for individual rats following each of the six intra-MR injections of the drugs described above. The order of drug injection was randomized among rats.

RESULTS

The results of Experiment 3 are shown in Fig. 5 where activity scores are plotted across time (12 min bins) following each intra-MR drug injection. It can be seen that muscimol, but not the other treatments, produced a large increase in locomotor activity. A 6×10 (drug × time bin) ANOVA with repeated measures on both factors conducted on scores from the 2 hour post-injection period indicated significant effects for time, $F(9,240)=7.45$, $p<0.001$, drug, $F(5,240)=114.04$, $p<0.001$, and a time × drug interaction, $F(45,240)=2.93$, $p<0.001$. Post hoc comparisons revealed that the hyperactivity produced by muscimol injections was responsible for all of the significant effects.

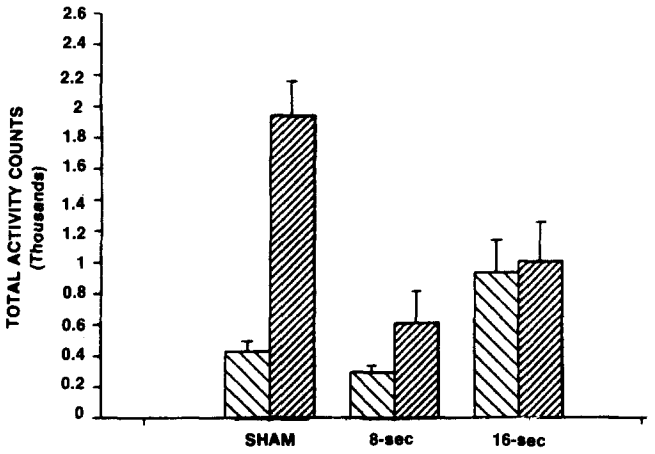

FIG. 7. Locomotor activity following intracranial injections of saline or muscimol for sham operated control animals and for subjects with 8 or 16 sec electrolytic MR lesions. Wide striped column: saline, narrow striped column: muscimol.

DISCUSSION

The results of Experiment 3 suggest that intra-MR injections of serotonin and fenfluramine fail to reproduce the hyperactivity seen after injections of muscimol. These results are in agreement with those of Fink and Morgenstern [11] who reported that intra-MR injections of d-lysergic acid diethylamide (LSD) fail to increase spontaneous activity. Although these results must be treated with some caution in the absence of biochemical or electrophysiological confirmation of an action on serotonergic cells, the fact that a variety of serotonergic agonists fail to produce hyperactivity following injections into the MR clearly fails to support the notion that acute reductions in the firing rate of serotonergic cells results in hyperactivity.

EXPERIMENT 4

The results of the first three experiments suggest that the hyperactivity resulting from injections of muscimol into the MR does not result from an inhibition of serotonergic cells. It is possible, therefore, that the effect might result either from an action on nonserotonergic cells within the vicinity of the MR, or from the diffusion of muscimol to an active site outside the MR. The latter possibility gains plausability from the fact that injections of muscimol into sites adjacent to the MR, such as the nucleus reticularis tegmenti pontis [8], and the ventral tegmental area [3] (but see [19]) may increase activity. The current experiment was thus designed to provide evidence as to whether muscimol injected into the MR is producing hyperactivity as a result of diffusion outside the region of the MR. In order to do this, we prepared animals with electrolytic lesions of the MR. These subjects were then implanted with cannulae terminating in the lesion cavity. If muscimol is working within the MR itself, the locomotor response should be greatly attenuated in these subjects.

METHOD

Surgery

Twenty rats served as subjects and were divided into three surgical groups: Les0, n=8; Les8, n=5; and Les16, n=7. Under pentobarbital anesthesia (50 mg/kg, IP), a stainless steel electrode, 0.23 mm in diameter with 0.5 mm of the tip uninsulated, was centered laterally on the superior sagittal sinus. The sinus was retracted and the electrode was lowered into the brain at coordinates AP: −0.3, H: −4.3 [29]. A 1-mA current was then passed from the electrode to a rectal cathode for either 8 (Les8) or 16 (Les16) seconds. The Les0 group was treated in a similar manner with the exception that after the burr hole was made in the skull the electrode was not lowered into the brain. Following 12 days of post-operative recovery, each rat underwent MR cannulation as described in Experiment 1.

Procedure

Photocell cage sessions, as described in Experiment 1, began 8 days after cannulation. Locomotor activity was measured following saline, then 3 days later after muscimol. Histology was conducted as in Experiment 1.

RESULTS

Histological examination revealed that all cannulae terminated either within the MR or within the cavity of the MR lesions. All of the electrolytic lesions severely damaged the MR. The 16 sec lesions were only slightly larger than the 8 sec lesions and extended a small amount outside the bounds of the MR to damage the region under the rostral pole of the dorsal tegmental nuclei and, in some animals, the very caudal portion of the ventral tegmental area. An example of the appearance of a typical 16 sec lesion is shown in Fig. 6.

The mean total activity scores for each group following saline and muscimol are shown in Fig. 7. Examination of the figure suggests that the MR lesions markedly reduced or eliminated the muscimol effect. A 3×2 (lesion size × muscimol/saline) repeated measures ANOVA conducted on the total 2 hour post-injection activity levels indicated significant effects of lesion size, $F(2,34)=6.16$, $p<0.004$, and of muscimol treatment, $F(1,34)=19.56$, $p<0.0001$, as well as a significant interaction, $F(2,34)=8.51$, $p<0.001$. The significant interaction term reflects the reduction in the size of the muscimol response seen in animals with electrolytic MR lesions. Analysis of simple main effects indicated that muscimol injections produced a significant ($p<0.001$) increase in activity in sham operated animals, but not in those with either 8 or 16 sec lesions. Analysis of the data obtained during the one hour habituation period (data not shown) indicated a significant effect of MR lesions, $F(2,34)=4.44$, $p<0.01$, and post hoc comparisons revealed that subjects with 16 sec lesions were more active than the other two groups ($p<0.05$). Although the 8 sec lesion animals tended to be more active than control subjects, this difference did not attain statistical significance ($p<0.1$).

DISCUSSION

Experiment 4 demonstrates that electrolytic lesions of the MR eliminate or greatly attenuate the hyperactivity produced by injections of muscimol. These results are, of course, compatible with the notion that muscimol is producing hyperactivity through an action within the immediate vicinity of the MR, a conclusion which is in agreement with the short latency of the muscimol effect (cf. Fig. 5). The possibility cannot be excluded that the glial scar formed at the lesion site might have interfered with the diffusion of muscimol, but it is interesting to note that in preliminary

studies we have found that the hyperactivity produced by injecting morphine into the MR [21] is not eliminated in rats with electrolytic MR lesions, suggesting that morphine injected into the MR produces hyperactivity at least in part as a result of diffusion to other structures. The failure of an electrolytic lesion to interfere with diffusion of morphine makes it less plausible that the diffusion of muscimol would be attenuated.

GENERAL DISCUSSION

The results of the current experiments provide no support for the notion that inhibition of serotonergic mechanisms underlies the hyperactivity seen after injections of muscimol into the MR. Muscimol-induced hyperactivity was not altered after large depletions of serotonin produced either by systemic treatment with PCPA or by intra-MR injections of 5,7-DHT. Furthermore, intra-MR injections of serotonin or fenfluramine, which would be expected to decrease the firing rate of serotonergic cells [1, 2, 33], failed to produce hyperactivity. The conclusion that inhibition of serotonergic cells does not underly the muscimol effect is, of course, consistent with numerous reports that chronic depletion of serotonin fails to increase locomotor activity in short duration tests [4, 13, 15, 16, 25, 26].

The reason for the discrepancies between the current report and that of Sainati and Lorens [31] is unclear. It is possible that the injections of 5,7-DHT made by these authors into the ventral tegmentum or the lateral ventricle may have produced some unmeasured neurochemical effect other than serotonin depletion. Indeed, these authors themselves found that electrolytic lesions of the ventral tegmental nuclei failed to attenuate the muscimol effect even though they produced serotonin depletions of about the same magnitude as those seen after their 5,7-DHT injections into the ventral tegmentum [31]. Combined with the current results, these findings demonstrate that it is possible to deplete serotonin by a variety of means without altering muscimol-induced hyperactivity. It is interesting to note that certain effects produced by injections of muscimol into the dorsal raphe nucleus have also been shown to be unaffected by serotonin depletion [7,30].

Since Experiments 1–3 provide strong evidence against serotonergic involvement in the effects of intra-MR muscimol injections, the possibility must be considered that the muscimol might be acting to produce hyperactivity by diffusing to a distant site. The plausability of this notion is enhanced by the fact that hyperactivity has been reported following injections of muscimol or GABA into both the ventral tegmental area [3] and the nucleus reticularis tegmenti pontis [8], both of which structures are fairly close to the MR. Experiment 4, however, provided some evidence against the role of structures outside the MR in mediating the effects of muscimol by demonstrating that injections of muscimol into the cavity of an electrolytic MR lesion failed to produce an increase in activity. Although suggestive, these results should be interpreted with some caution since it is remotely possible that the glial scar at the injection site might have impeded the diffusion of muscimol. In other studies (in preparation) we have found that the hyperactivity produced by muscimol injections into the ventral tegmental area is of much smaller magnitude than that seen after intra-MR injections, further supporting the notion that the MR itself is the site of muscimol's effects on activity.

In conclusion, the simplest hypothesis that can be offered to account for the current results is that intra-MR injections of muscimol produce hyperactivity by inhibiting nonserotonergic cells within the immediate vicinity of the MR. This hypothesis is in excellent agreement with the results of anatomical studies which have demonstrated that most of the cells in the MR are nonserotonergic [24,28] and with the results of excitotoxin studies which have suggested that destruction of nonserotonergic MR cells produces hyperactivity [4].

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