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Comparative Studies of Locomotor Behavior Following Microinjections of Muscimol Into Various Sites in the Paramedian Tegmentum

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WIRTSHAFTER, D. AND M. A. KLITENICK. *Comparative studies of locomotor behavior following microinjections of muscimol into various sites in the paramedian tegmentum.* PHARMACOL BIOCHEM BEHAV **32**(3) 625–628, 1989.—Microinjections of various doses of muscimol into the median raphe nucleus, the dorsal raphe nucleus or the caudal portion of the ventral tegmental area elicited dose-dependent increases in locomotor activity. In contrast, injections into the rostral portion of the ventral tegmental area or the midline pontine tegmentum caudal to the median raphe were ineffective. Lower doses of muscimol were required to produce hyperactivity after injections into the median raphe than after injections into any of the other sites. These findings suggest that the median raphe nucleus is the most sensitive site in the paramedian tegmentum for the elicitation of hyperactivity by muscimol.

Muscimol GABA Locomotor activity Median raphe Dorsal raphe Ventral tegmental area

SEVERAL recent studies have demonstrated that marked increases in locomotor activity can be produced by injections of the GABA-A agonist muscimol into the median raphe nucleus (MR) (7, 11, 12, 14, 15). The hyperactivity produced by intra-MR muscimol does not appear to be dependent on serotonergic mechanisms (7,14) and it is relatively insensitive to blockade by the dopamine antagonist haloperidol (15). The behavioral stimulation produced by intra-MR muscimol injections is not restricted to locomotor activity as these treatments also result in robust increases in food and water intake in nondeprived rats (6).

In order to correctly interpret the actions of muscimol injected into the MR, it is important to ascertain whether the muscimol is acting in the MR itself or whether it is producing its behavioral effects as a result of diffusion to another site. The practical importance of this problem can be seen from the fact that hyperactivity has been reported following injections of muscimol into the dorsal raphe nucleus (10,12) and the ventral tegmental area (1), both of which structures are closely proximate to the MR.

In the current study we attempted to more adequately localize the site of muscimol's actions by comparing the locomotor responses to injections in the MR, the dorsal raphe (DR), the rostral and caudal portions of the ventral tegmental area (VTA) and the pontine tegmentum caudal to the MR (PT).

METHOD

Subjects

Subjects were 32 male Sprague-Dawley rats obtained from a colony maintained by the University of Illinois at Chicago. The rats weighed 280–320 g at the time of surgery and were housed individually in wire-mesh cages on a 12:12 hr light dark cycle.

Surgery

Surgery was conducted under sodium pentobarbital anesthesia (50 mg/kg) using standard stereotaxic techniques. Twenty-two gauge stainless steel guide cannulae were implanted so as to terminate 2 mm above either the MR (AP: −0.2, H: −2.3) (8), the DR (AP: −0.2, H: −0.5), the rostral VTA (AP: 2.2, H; −2.0), the caudal VTA (AP: 1.0, H: −2.1), or the midline pontine tegmentum (AP: −1.4, H: −2.8). The guide cannulae were lowered on the midline following retraction of the superior sagittal sinus (13) and were attached to the skull with dental cement. A 28-gauge stainless steel obdurator was then inserted in the guide cannula and extended 2 mm beyond its tip. Subjects were allowed a seven-day period to recover from surgery during which they were handled daily.

Apparatus

Locomotor activity levels were measured in one of four identical infrared photocell boxes measuring 71.5 × 71.5 × 27 cm. Four infraed beams were located 3.5 cm above the floor to detect horizontal movements. The box was painted black and lighting was provided by overhead fluorescent fixtures.

Procedure

Each rat received four tests of locomotor activity beginning on

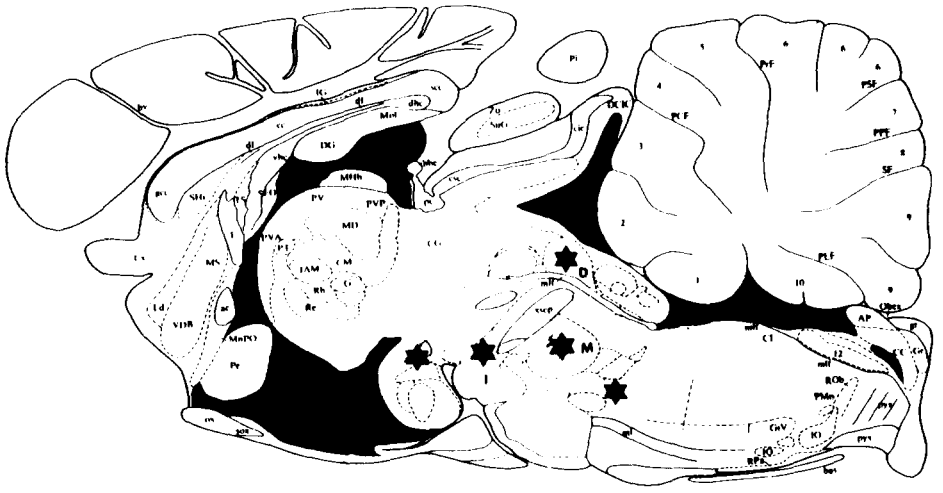

FIG. 1. Schematic illustration in the sagittal plane showing the locations of cannula tips in the (left to right) rostral VTA, caudal VTA, median raphe, dorsal raphe, and caudal pontine tegmentum. Abbreviations: D—dorsal raphe, M—median raphe, I—interpeduncular nucleus.

day 8 following surgery. Animals were individually placed in the activity cages and allowed to habituate to them for one hour. The rats were then gently taken from the cages and their obdurators removed and replaced by 28-gauge stainless steel injection cannula trimmed so as to extend 2 mm beyond the end of the guide. The injection cannula was connected by a length of polyethlylene tubing to a motor driven Hamilton microsyringe which delivered the infusate at a rate of 0.25 μl/min. The injection cannula was left in place for 30 sec following the completion of the injections at which time it was removed, the obdurator replaced, and the subject returned to its activity box for a further two hours. Rats received injections of either normal saline or of 6.25, 25, or 100 ng of muscimol. All injections were made in a volume of 0.25 μl except for the 100 ng of muscimol which was injected in a volume of 0.5 μl. Injections were made in a randomized order in individual animals and at least three days separated consecutive treatments.

Histology

Following the completion of behavioral studies, rats were perfused, under deep pentobarbital anesthesia, with normal saline followed by 10% formalin. Sixty-four micron frozen sections were then prepared through the injection sites and stained with cresyl violet.

RESULTS

Histological evaluation indicated that all of the cannulae terminated in the appropriate target structures (Fig. 1). MR cannulae terminated within the MR at, or slightly rostral to, the level of the ventral tegmental nuclei of Gudden, similar to the placements we have described in previous reports (14–17). Caudal VTA placements were located immediately dorsal to the central portion of the interpeduncular nucleus (IPN) [c.f., (17)], whereas rostral VTA placements were centered rostral to the IPN, at or behind the level of the caudal pole of the mamillary bodies. PT placements were located within, or just ventral to, the pontine raphe nucleus. Total activity scores across the 2-hr postinjeciton periods are shown in Fig. 2 where it can be seen that injections into the MR produced larger effects on activity than did injections into the other four sites. Analysis of this data by means of a multivariate analysis of variance indicated a significant interaction between cannula placement and drug dose, $F(12,66)=5.72$, $p<0.001$. Analysis of contrasts indicated that all three doses of muscimol injected into the MR resulted in significant hyperactivity relative to saline baselines ($p<0.001$). In contrast, only the 100 ng dose produced significant effects in animals with cannulae in the DR or caudal VTA ($p<0.001$). No significant effects were seen with injections into the rostral VTA or the pontine tegmentum ($p>0.5$). Furthermore, animals with cannulae in the MR showed significantly larger increases in activity over baseline in response to all three doses of muscimol than did animals with any of the other placements ($0.001>p>0.05$).

The time course of the response to muscimol also varied depending on the cannulae placements (Fig. 3). In order to analyze these data, the latency to the occurrence of half maximal hyperactivity following injections of 100 ng of muscimol was calculated for each subject in the MR, DR and caudal VTA groups. This measure of rise time yielded values of 4.4 ± 0.4 min for MR animals, 9.2 ± 2.4 min for DR subjects, and 38.8 ± 7.0 min for VTA animals. Analysis of this data by means of a one-way

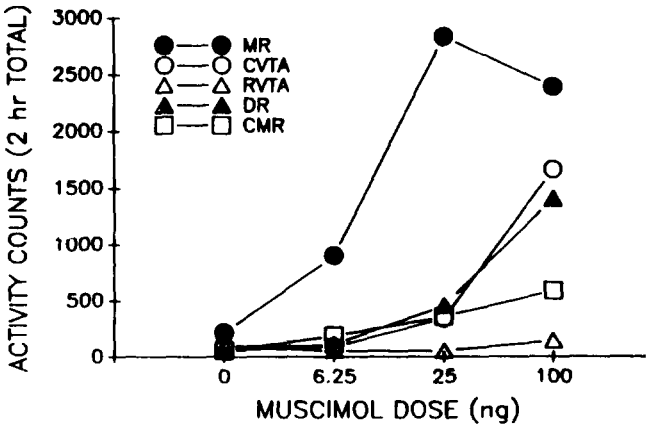

FIG. 2. Cumulated activity counts following injections of various doses of muscimol into the median raphe (●), the dorsal raphe (▲), the caudal VTA (○), the rostral VTA (△), and the caudal pontine tegmentum (□).

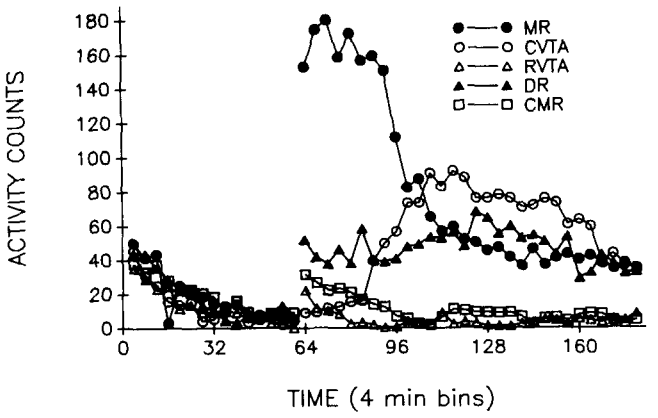

FIG. 3. Locomotor activity in four-min time bins during the one-hour habituation period and the two-hour test period following injections of muscimol into the median raphe (●), the dorsal raphe (▲), the caudal VTA (○), the rostral VTA (△), and the caudal pontine tegmentum (□).

planned comparisons analysis of variance indicated that the latencies were significantly longer with injections into the caudal VTA than with injections into the MR or DR, $F(2,16)=71.98$, $p<0.001$. In contrast, latencies did not differ significantly between subjects with MR and DR cannulae ($p>0.1$).

DISCUSSION

The current results confirm previous reports of hyperactivity following injections of muscimol into the MR (7, 11, 12, 14, 15), the DR (10,12) and the VTA (1). In contrast, injections into the pontine tegmentum caudal to the MR were without effect at the doses utilized. Injections into the MR produced significant effects at doses lower than those required at any of the other sites, and the maximal responses seen after injections into the MR were again larger than those seen after injections into the other areas studied. Taken together, these findings support the conclusion that muscimol injected into the MR produces hyperactivity as a result of an action on local cell bodies rather than by diffusion to a distant site. These results are in agreement with previous findings that infusions of muscimol, following electrolytic or ibotenic acid lesions of the MR, fail to produce hyperactivity (7,14). In other studies we have found that injections of muscimol into the MR also result in a larger increase in food and water intake than do injections into the VTA or the DR (6).

Although the possibility was not examined in the present study, it is very unlikely that diffusion to structures lateral to the MR could play a role in the hyperactivity. Injections of muscimol into the midbrain reticular formation lateral to the MR have been found to produce significant hypoactivity (3), an effect opposite to that seen after MR injections. Furthermore, electrolytic lesions of the ventral tegmental nuclei of Gudden, which are located dorsolateral to the MR, fail to alter the hyperactivity produced by muscimol injections in the MR (11).

The current results raise the possibility that the hypermotility seen after injections of muscimol into the VTA or the DR might, in fact, result from diffusion of the drug into the MR. The evidence for this possibility is strongest for the case of the VTA. Hyperactivity is seen after injections into the caudal VTA, which is adjacent to the MR, but not after injections near the rostral VTA, which is more distant. In contrast, picrotoxin, which does not appear to alter activity when injected into the MR (12), produces hyperactivity when applied to the rostral, but not the caudal, VTA (1). Furthermore, the effects of muscimol injected into the MR are immediate in onset, whereas those seen after injections in the VTA are delayed by about 20 min, an observation also reported by other workers (1). These results are, of course, compatible with the notion that muscimol injected into the VTA may be producing its effects as a result of diffusion to the MR, a possibility which could be tested by examining whether MR lesions alter the behavioral response to muscimol applied into the caudal VTA. The current results, in agreement with those of other workers (11), demonstrate that the DR is a less sensitive site than the MR for producing hyperactivity with muscimol injections. The mild hyperactivity which was produced by these injections was not significantly delayed relative to that produced by MR injections. In contrast, Sainati and Lorens (11) have reported a more rapid onset of hyperactivity with injections into the MR than the DR. This observation must be interpreted carefully, however, since it was made in animals who received acute injections of muscimol under ether anesthesia. It is possible that the mild hyperactivity produced by injections in the DR may have been more easily suppressed by some residual effect of the anesthetic than was the more dramatic hyperactivity produced by injections in the MR. Thus, although muscimol injected into the DR might be producing hyperactivity as a result of diffusion into the MR, the possibility cannot be ruled out that the DR is merely less sensitive to the effects of muscimol than is the MR. It is interesting to note that much larger increases in locomotor activity are seen after lesions of the MR than the DR (2, 4, 5).

In summary, the current findings demonstrate that the MR is the most sensitive site in the paramedian tegmentum for the elicitation of hyperactivity by injections of muscimol. In other studies, we have demonstrated that this statement also holds true for the hyperactivity elicited by injections of a variety of excitatory amino acid antagonists (17). Hyperactivity has also been reported after intra-MR injections of opiate agonists (in preparation), and clonidine (9), and acute intra-MR injections of kainic acid have been shown to reduce activity levels (16). These results indicate that the MR may play an important role in the control of behavioral activation and suggest that this nucleus is worthy of a greater amount of experimental attention than it has heretofore received.

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REFERENCES

1. Arnt, J.; Scheel-Kruger, J. GABA in the ventral tegmental area: differential regional effects on locomotion, aggression and food intake after microinjection of GABA agonists and antagonists. Life Sci. 25:1351–1360; 1979.
2. Asin, K. E.; Wirtshafter, D.; Kent, E. W. Straight alley acquisition and extinction and open field activity following discrete electrolytic lesions of the mesencephalic raphe nuclei. Behav. Neural Biol. 25:242–256; 1979.
3. Brudzynski, S. M.; Houghton, P. E.; Brownlee, R. D.; Mogenson, G. J. Involvement of neuronal cell bodies of the mesencephalic locomotor region in the initiation of locomotor activity of freely behaving rats. Brain Res. Bull. 16:377–381; 1986.
4. Geyer, M. A.; Puerto, A.; Menkes, D. B.; Segal, D. S.; Mandell, A. J. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. Brain Res. 106:160–177; 1980.
5. Jacobs, B. L.; Cohen, A. Differential behavioral effects of lesions of the median or dorsal raphenuclei in rats: open field and pain-elicited aggression. J. Comp. Physiol. Psychol. 90:102–108; 1976.

6. Klitenick, M. A.; Wirtshafter, D. Comparative studies of ingestive behavior following injections of muscimol into the median or dorsal raphe nuclei or the ventral tegmental area. Life Sci. 42:775–782; 1988.
7. Paris, J. M.; Lorens, S. A. Intra-median raphe infusions of muscimol and the substance P analogue DiMe-C7 produce hyperactivity: role of serotonin neurons. Behav. Brain Res. 26:139–151; 1987.
8. Pellegrino, L. J.; Cushman, A. S. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Crofts; 1967.
9. Plaznik, A.; Danysz, W.; Kostowski, W.; Bidzinski, A.; Hauptman, M. Interaction between noradrenergic and serotonergic brain systems as evidenced by behavioral and biochemical effects of microinjections of adrenergic agonists and antagonists into the median raphe nucleus. Pharmacol. Biochem. Behav. 19:27–32; 1983.
10. Przewiocka, B.; Stala, L.; Scheel-Kruger, J. Evidence that GABA in the nucleus raphe dorsalis induces stimulation of locomotor activity and eating behavior. Life Sci. 25:937–946; 1979.
11. Sainati, S.; Lorens, S. A. Intra-raphe muscimol-induced hyperactivity depends on ascending serotonergic projections. Pharmacol. Biochem. Behav. 17:973–986; 1982.
12. Sainati, S.; Lorens, S. A. Intra-raphe benzodiazepines enhance rat locomotor activity: Interactions with GABA. Pharmacol. Biochem. Behav. 18:407–414; 1983.
13. Wirtshafter, D.; Asin, K. E.; Kent, E. W. A simple technique for midline stereotaxic surgery in the rat. Physiol. Behav. 23:409–410; 1979.
14. Wirtshafter, D.; Klitenick, M. A.; Asin, K. E. Evidence against serotonin involvement in the hyperactivity produced by injections of muscimol into the median raphe nucleus. Pharmacol. Biochem. Behav. 27:45–52; 1987.
15. Wirtshafter, D.; Klitenick, M. A.; Asin, K. E. Is dopamine involved in the hyperactivity produced by injections of muscimol into the median raphe nucleus? Pharmacol. Biochem. Behav. 30:577–583; 1988.
16. Wirtshafter, D.; McWilliams, C. Suppression of locomotor activity produced by acute injections of kainic acid into the median raphe nucleus. Brain Res. 408:349–352; 1987.
17. Wirtshafter, D.; Trifunovic, R.; Krebs, J. C. Behavioral and biochemical evidence for a functional role of excitatory amino acids in the median raphe nucleus of the rat. Brain Res. 482:225–234; 1989.