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RAPID COMMUNICATION

Dorsal Hippocampal Lesion Abolishes the Response to FG-7142 in the Rat

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LIPSKA, B. K., G. E. JASKIW AND D. R. WEINBERGER. *Dorsal hippocampal lesion abolishes the response to FG-7142 in the rat.* PHARMACOL BIOCHEM BEHAV **40**(1) 169–172, 1991.—The effects of the anxiogenic β-carboline FG-7142 (15 mg/kg IP) on exploratory locomotor activity were assessed in rats with sham or ibotenic acid (IA) lesions of the dorsal or ventral hippocampus. FG-7142 reduced exploratory activity similarly in control animals as well as in those with IA lesions of the ventral hippocampus. In contrast, FG-7142 had no effect on rats with dorsal hippocampal lesions. The results suggest that the dorsal hippocampus plays a unique role in FG-7142-mediated attenuation of locomotor exploration. Other studies suggest that serotonergic systems may mediate these properties of FG-7142.

Ibotenic acid lesion Dorsal hippocampus Ventral hippocampus FG-7142 Locomotion Exploration

THE mechanism by which the anxiogenic β-carboline FG-7142 reduces exploratory locomotor activity in rats (6,14) is not known. The ability of FG-7142 to enhance DA release within the medial prefrontal cortex (MPFC) (28) and the role of MPFC DA systems in attenuating subcortical DA turnover (24), prompted us to hypothesize that actions within the MPFC were critical to FG-7142-induced attenuation of locomotor exploration (14). However, the observation that ibotenic acid (IA) lesions of the MPFC potentiated FG-7142-induced reductions in exploration (14) indicates that some other brain region must be involved in the effects of FG-7142 on exploratory behavior.

It has been recently demonstrated that FG-7142 reduces serotonergic (5HT) transmission in the dorsal hippocampus by interacting with γ-aminobutyric-benzodiazepine receptor (GABA A/BZR) sites presumably located on serotonergic (5HT) terminals from raphe nuclei (18,19). The hippocampus, and particularly the 5HT projections to the dorsal hippocampus, have been strongly implicated in the control of locomotor exploration. Accordingly, we postulated that the dorsal hippocampus (DH) might mediate FG-7142-induced reductions in exploratory activity, and tested the latter by evaluating the effects of FG-7142 in rats with IA lesions of the dorsal hippocampus. To assess the anatomic specificity of the results, similar testing was conducted in rats with ventral hippocampal (VH) lesions.

METHOD

Male Sprague-Dawley rats (Zivic Miller Labs, 200–250 g) were housed 3 to a cage with free access to food and water under a light cycle maintained between 7:00 a.m. and 7:00 p.m. After induction of anesthesia with Equithesin 3 cc/kg (IP), animals were placed in a Kopf stereotaxic instrument with the tooth bar at 2.5 mm below the interaural line. Dorsal hippocampal lesions were induced by injection of IA (5 μg/0.5 μl over 2.5 min, Sigma Chemical Co.) or vehicle (0.1 M phosphate-buffered saline, pH 7.4) bilaterally by infusion pump (Harvard Apparatus) through stainless steel 26-gauge cannulae at the coordinates: AP −3.0 mm, ML ±2.2 mm, VD −3.9 mm (22). For VH lesions, two injections of vehicle or IA (6 μg/0.6 μl over 3 min) were made bilaterally at the coordinates: AP −4.4 mm, ML ±5.0 mm, VD −8.0 and 6.0 mm. Cannulae remained in place for 5 min after the end of each infusion. Six rats from each lesioned group were anesthetized with sodium pentobarbital 14 days postoperatively and decapitated. Frozen brains were used for preparation of cresyl violet stained sections.

Six weeks after the surgery rats were brought to the testing area in their home cages and acclimatized overnight. At 10:30 a.m., FG-7142 (15 mg/kg, suspended in distilled water with 3 drops of Tween 80/10 ml) or vehicle (VEH) were injected IP. Twenty minutes later, rats were placed in clear Plexiglas activity monitors (42 × 42 × 30 cm) (Omnitech model RXYZCM 16) (25). Spontaneous locomotor activity was measured during a 60-min period at 15-min intervals. Distance traveled was analysed using a three-factor ANOVA with drug (FG-7142 or VEH) and lesion status (ibotenic acid lesion or sham) as single factors and time as repeated measure. Newman-Keuls tests were used for post hoc comparisons.

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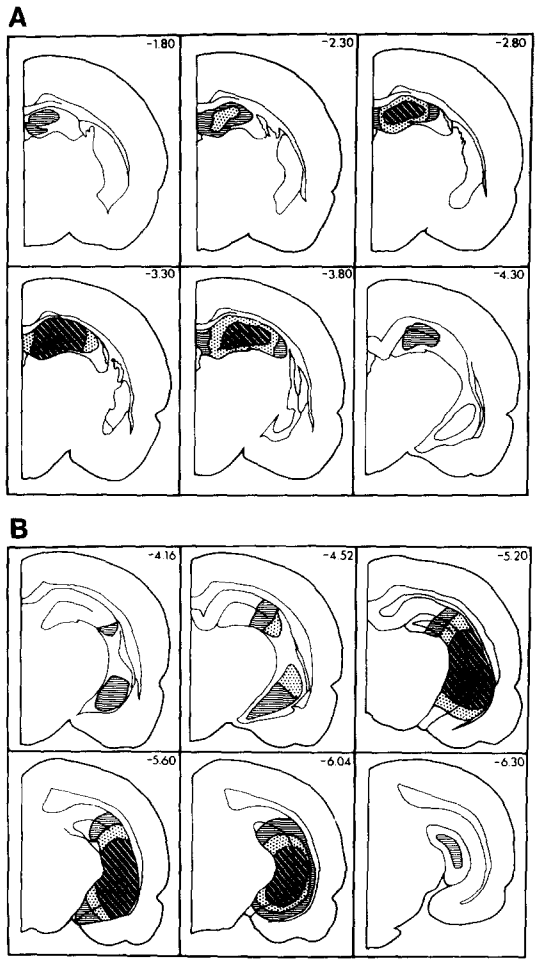

FIG. 1. Lesion boundaries shown at the coronal drawings and defined as the area of neuronal absence. Determined from cresyl violet stained coronal sections from 16 rats with IA lesions of (A) dorsal, (B) ventral hippocampus. Horizontal bars and the diagonal bars indicate the largest and smallest lesions, respectively. Stippling indicates the area encompassing the lesion boundaries in 10 rats. The numbers show the distance of the plate from bregma (mm).

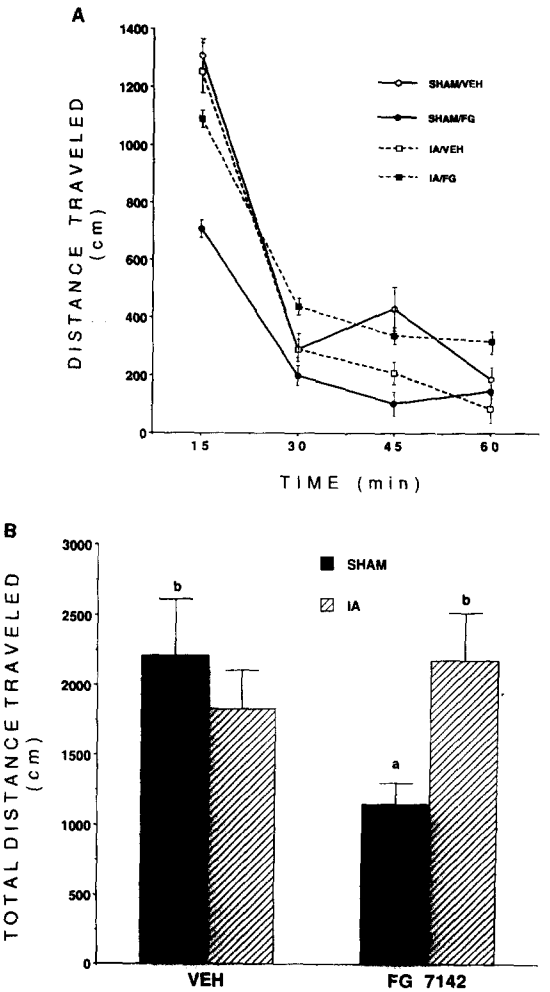

FIG. 2. (A) Time course of distance traveled over 60 minutes by the four groups (N = 10 per group), starting 20 min after injection of either FG-7142 (15 mg/kg IP) or vehicle (VEH) (distilled water with 3 drops of Tween 80). Rats received either sham or ibotenic acid (IA) lesions of the dorsal hippocampus 6 weeks earlier. (B) Total distance traveled over 60 minutes by the four groups (means ± SEM). IA/FG rats were markedly hyperactive as compared to sham/FG group (a significantly different from b, $p<0.05$) and their locomotor activity did not differ from that of vehicle-treated animals.

RESULTS

Cresyl violet stains revealed neuronal loss and gliosis but no cavitation in the IA-lesioned DH hippocampus in fields CA1 and CA4. Damage to adjacent structures or the VH hippocampus was not seen in any of these rats (Fig. 1A). As expected, however, neurons were absent from most of the area comprising the VH and the subiculum in the VH lesioned group. As is evident from the figure (Fig. 1B), the DH and VH lesions did not overlap.

Within the DH group a three-way ANOVA of the distance traveled did not reveal main effects of lesion (LSN) or treatment (RX), but LSN × RX as well as RX × TIME interactions were significant ($F=5.4$, $p<0.03$ and $F=3.2$, $p<0.03$, respectively). The lesion × drug × time interaction did not reach significance ($F=0.23$, $p<0.8$). Post hoc tests demonstrated that the distance traveled by sham/FG rats was reduced in comparison to the sham/VEH animals ($p<0.05$). This effect was abolished in animals with IA lesions of the DH (Fig. 2). The total distance traveled by DH IA/FG rats did not differ from that of the IA/VEH group, although it was significantly greater than that of sham/FG animals ($p<0.05$).

Within the VH group, a three-way ANOVA demonstrated a significant main RX effect ($F=13.5$, $p<0.001$) and a significant RX × TIME interaction ($F=8.4$, $p<0.0001$) on distance traveled. The main LSN effect ($F=0.2$, $p<0.7$) and LSN × RX × TIME interaction ($F=0.5$, $p<0.7$) were not significant. Post hoc comparisons revealed that the distance traveled by sham/FG rats was reduced compared to the sham/VEH group ($p<0.05$). While IA/VEH rats showed more intense exploratory locomotion than sham/VEH animals, the distance traveled by the IA/FG group was reduced relative to either the IA/VEH or sham/VEH groups ($p<0.05$), and was similar to that of the sham/FG rats (Fig. 3).

DISCUSSION

We have investigated the effect of FG-7142 on locomotor exploration in rats with ibotenic acid lesions of the dorsal or ventral hippocampus. As expected, in both sham-operated groups

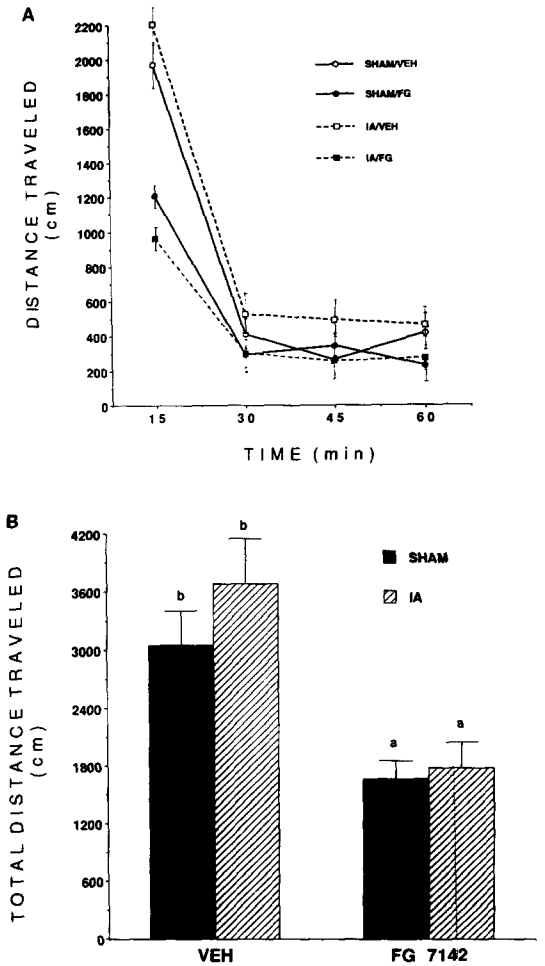

FIG. 3. (A) Time course of distance traveled over 60 minutes by the four groups (N = 10 per group), starting 20 min after injection of either FG-7142 (15 mg/kg IP) or vehicle (VEH) (distilled water with 3 drops of Tween 80). Rats received either sham or ibotenic acid (IA) lesions of the ventral hippocampus 6 weeks earlier. (B) Total distance traveled over 60 minutes by the four groups (means ± SEM). FG-7142 reduced locomotor activity of both sham and lesioned rats to the same degree (a significantly different from b, $p<0.05$).

FG-7142 attenuated the initial intense phase of exploratory activity. Their activity was reduced to a similar extent (by approximately 50%), despite the fact that it differed at baseline between the groups. We reported before the same reduction in rats with sham MPFC lesions (14).

Our experience shows that the normal variations in baseline spontaneous activity in control rats do not affect the response to FG-7142 (G.J., B.L., unpublished observation).

FG-7142 also suppressed the hyperactive locomotor behavior in rats with IA lesion of the ventral hippocampus, but had no effect in dorsal hippocampally lesioned animals.

The capability of FG-7142 to inhibit the locomotor activity in IA VH-lesioned rats suggests that the ventral hippocampus is not a principle site of action for FG-7142, at least as far as modulation of exploratory locomotion in the rat is concerned. As we previously reported (14), the destruction of the MPFC in the rat did not abolish the inhibition of locomotion in an open field, either. In contrast, the dorsal hippocampus seems to play an important role in mediating the FG-7142-induced changes of exploratory locomotor activity, while the mechanism of this is not known yet.

FG-7142 has been shown to selectively increase MPFC DA turnover and to exert an inhibitory effect on DA release in nucleus accumbens and corpus striatum (28). As both these regions have been implicated in control of exploratory activity, one might assume that attenuation of exploration by FG-7142 may be linked to its action in the mesolimbic system via corticofugal projections of MPFC. However, as we previously reported, MPFC lesion did not abolish FG-7142-induced attenuation of locomotion; on the contrary, the lesion amplified it. Accordingly, we concluded that the observed behavioral response to FG-7142 in intact rats does not depend on an enhancement of MPFC DA transmission.

Recent studies on the pharmacology of FG-7142 suggest that some of its effects may be mediated through serotonergic neural systems. FG-7142 is reported to exert anxiogenic effects in several species, including humans (5,23). In this respect it acts in an opposite way to benzodiazepines and is referred to as a partial inverse agonist at the benzodiazepine receptor site (2). FG-7142 has recently been shown to bind to GABA A/BZR receptors located presumably presynaptically on serotonergic neurons and to modulate release of serotonin (16,17). The medial prefrontal cortex, as well as the dorsal and ventral hippocampus, are all densely innervated by serotonergic neurons (1, 7, 15, 21, 26). There are, however, differences between these regions in the origin of the serotonergic input. The major component of MPFC serotonin innervation arises from the dorsal raphe nuclei (15). Also, as pointed out by Gray (7) and Moore (20), neurons that innervate the ventral hippocampus originate mostly in the dorsal raphe and reach ventral hippocampus through the ventral amygdaloid bundle, whereas fibers from median raphe reach the dorsal hipocampus taking both the fimbria-fornix route and supracollosal route. These observations indicate that serotonin neurons of the raphe nuclei provide a highly organized and regionally differentiated innervation of the prefrontal cortex and hippocampal formation.

The differential regional serotonergic innervation seems to be paralleled by regional differences in the molecular constitution of GABA A/BZ receptors located on serotonergic terminals. DMCM, a potent GABA/BZR inverse agonist, reduced 5HT transmission via BZR in the dorsal hippocampus (18), as demonstrated also for FG-7142 (16), but did not alter serotonin release through GABA A/BZ receptor in the prefrontal cortex. As suggested by Costa (4) and Lista (18), serotonin fibers that terminate in the prefrontal cortex (and arise from the dorsal raphe lack GABA A/BZ sites for inverse agonists, while those neurons projecting to the dorsal hippocampus from median raphe nucleus possess recognition sites for benzodiazepine inverse agonists (18).

It is tempting to speculate that there may be a link between the ability of FG-7142 to bind to GABA A/BZR in the dorsal hippocampus and, therefore, to modulate serotonin release, and locomotor alterations observed following administration of the drug to intact rats. The role of brain serotonin in mediating locomotor activity has been the subject of considerable investigation. PCPA, a serotonin-depleting drug acting via inhibition of 5HT synthesis, was shown to induce hyperactivity mediated specifically by the hippocampus (3,13). Serotonergic neurons originating in the dorsal or median raphe appear to differentially modulate locomotor activity and DA systems in the rat. It has been reported that lesions specifically confined to the median raphe nucleus produce dramatic elevation of locomotor activity and marked depletion of hippocampal serotonin, whereas lesions of the dorsal raphe nucleus have no effect on activity (12,13). The dorsal raphe lesion caused the increase in DA utili-

zation in nucleus accumbens, but no effect in MPFC (8), whereas the lesion of median raphe resulted in enhancement of DA turnover in nucleus accumbens, and a reduction in DA utilization in MPFC (9).

It may be concluded from these studies that under certain experimental conditions the two neurotransmitter systems, DA and 5HT, may strongly interact in the brain and affect locomotion (10,11).

Whether the regulatory interactions occur in DA terminal fields, e.g., in the nucleus accumbens, which receives rich excitatory (glutamatergic) input from hippocampal formation (27) and generates motor behaviors, or in the cell bodies of origin of the dopaminergic innervation, ventral tegmental area, which receives serotonergic projections from raphe nuclei, remains to be explored.

Our results indicate a specific role of the dorsal hippocampus in mediating FG-7142-induced attenuation of exploratory locomotion. However, further experiments are required to characterize the mechanism of this regulation.

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