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BRIEF COMMUNICATION

Intracortical 5,7-Dihydroxytryptamine Depletes Brain Serotonin Concentrations Without Affecting Spontaneous Activity

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BLACK, R. S. AND R. G. ROBINSON. *Intracortical 5,7-dihydroxytryptamine depletes brain serotonin concentrations without affecting spontaneous activity.* PHARMACOL BIOCHEM BEHAV 22(2) 327–331, 1985.—Microinjections of either 2 or 4 μg of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) were made into either the right or left frontal cortex of male rats pretreated with desmethylimipramine. Although both the 2 and 4 μg doses produced significant depletions of serotonin concentrations in the cortex and median raphe, neither dose produced a significant increase in spontaneous activitity. This is in contrast to our findings with the noradrenergic neurotoxins, 6-hydroxydopamine and DSP-4, which produced hyperactivity following right but not left hemisphere injections. These findings do not rule out the involvement of 5-HT in the asymmetrically elicited hyperactivity but they do suggest some transmitter and neural pathway specificity to the lateralized response to cortical injury.

5,7-Dihydroxytryptamine Asymmetry Lateralization Serotonin Behavior

PREVIOUS studies have found that ligation of the right middle cerebral artery, or focal suction ablation of the right frontal cortex in the rat led to spontaneous hyperactivity and both cortical and subcortical depletions in brain catecholamine concentrations while identical lesions of the left frontal cortex did not [17, 18, 22, 24].

We have hypothesized that this asymmetry in the behavioral response to right versus left hemisphere lesions may result from the differential effect of these lesions on catecholaminergic neurons [25]. This suggestion is supported in part by the finding that low dose injections of the catecholamine neurotoxin 6-hydroxydopamine or the noradrenergic neurotoxin DSP-4 into the right frontal cortex produced spontaneous hyperactivity, while identical injections into the left hemisphere did not [12,26].

Norepinephrine (NE), however, is only one of the biogenic amine neurotransmitters found in the cerebral cortex. The terminal fields of serotonergic neurons have been shown to have the same widespread cortical distribution as NE [14,15]. We therefore wanted to determine whether injury to serotonergic neurons as produced by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) in combination with desmethylimipramine (DMI) [1] could lead to post-lesion hyperactivity.

METHOD

Sprague-Dawley male rats (weighing approximately 300 g) were housed individually for 3 weeks prior to operation in running wheel cages [20] with food and water freely available in a regular 12 hour light, 12 hour dark environment. Baseline activity as measured by 24 hour cyclometer readings and food and water intake were recorded for one week prior to surgery.

Desmethylimipramine (25 mg/kg) was given intraperitoneally one hour before the animal was injected with neurotoxin in order to prevent non-specific uptake into NE neurons [1]. Under chloral hydrate anesthesia (350 mg/kg) rats were placed in a stereotaxic apparatus and a 29-gauge cannula was lowered to 1.5 mm below the surface of the cortex and then retracted to 1.0 mm depth. All injections were made using an automated syringe with a constant rate of infusion of 0.3 μl/min between 8.5 and 9.0 mm anterior to ear bar zero, and 2.0 mm dorsal to plane zero. Doses of 2 μg and 4 μg of 5,7-DHT or vehicle were injected into either the right or left hemisphere in concentrations such that a 1 μl total injection volume was used.

At 30 days after surgery, the animals were sacrificed and their brains quickly removed, dissected over ice using a me-

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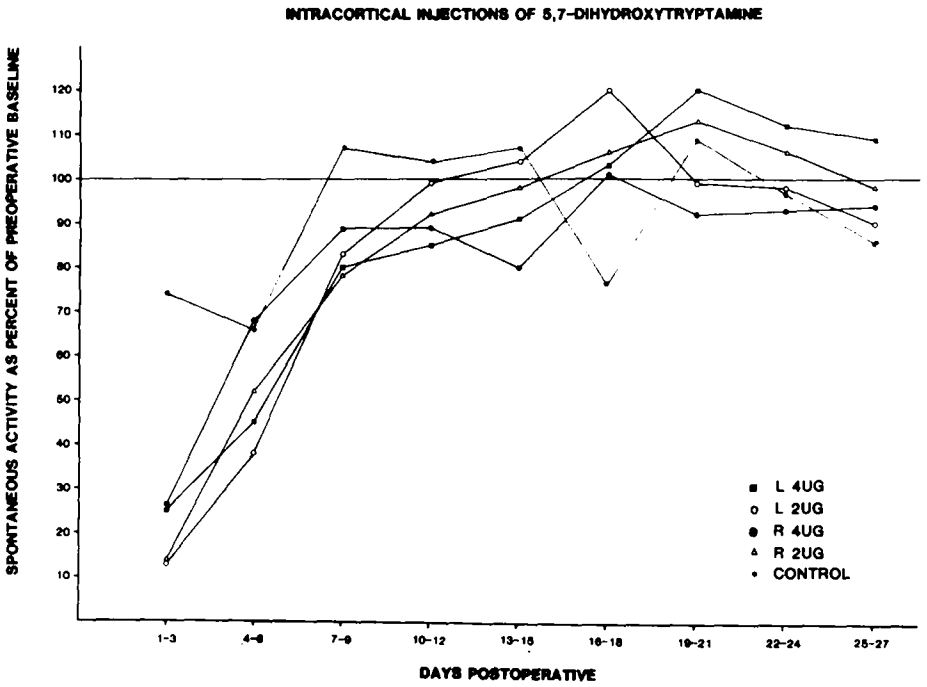

FIG. 1. Daily running wheel activity following intracortical injection of 5,7-dihydroxytryptamine expressed as percentage of preoperative baseline and averaged into 3 day means. The numbers of animals tested in each treatment group were: L 2 μg=4, L 4 μg=8, R 2 μg=4, R 4 μg=8, control=8. There were no significant differences in spontaneous activity between right or left hemisphere injected groups or among control, 2 μg or 4 μg dosage groups.

chanical brain slicing apparatus [6] as described in previous publications [11,12]. Brain samples were taken from the anterior and posterior cortex between the rhinal fissure and cingulate cortex, the substantia nigra, and the median raphe (i.e., the brainstem section was bisected at the midline and the ventral medial portion was taken as the median raphe). Samples were stored at −80°C and analyzed for norepinephrine, dopamine and serotonin concentrations using high pressure liquid chromatography with electrochemical detection (Bioanalytical Systems, LC-54, LaFayette, IN) as described in previous publications [11,12]. Briefly, samples were sonified 0.1% perchloric acid, centrifuged and 15 μl samples were injected onto a u-Bondapak C-18 reverse phase column by means of an automated sample injector (WISP 720B, Waters Instruments Co., Milford, MA) and eluted with 10% acetonitrile and 0.1% heptanesulfonic acid and buffered to a pH of 4.5 with sodium acetate-acetic acid. Concentrations were calculated using an integrator (Hewlett-Packard) with the method of internal standards.

Measures of biochemical concentrations for each biogenic amine in each brain region were analyzed with a mixed-model factorial analysis of variance. The between-subjects factors were dose (control, 2 μg and 4 μg) and injection location (left and right). The repeated measures factor was biogenic amine concentrations at both left and right hemispheres, or in the case of activity, ten 3 day measurement periods. In order to obtain a balanced design, the control subjects given left hemisphere vehicle injections were assigned to the left side of injection condition and the control subjects given right hemisphere vehicle injection were assigned to the right-side of injection condition.

RESULTS

During the first 2 weeks postoperative, animals receiving 2 or 4 μg of 5,7-DHT demonstrated a slow return of their spontaneous activity to baseline levels (Fig. 1). Repeated measures analysis of variance demonstrated no significant right versus left treatment differences, $F(1,22)=0.026$, $p>0.05$, or dose effects-control, 2 or 4 μg, $F(2,29)=0.19$, $p>0.05$ (Fig. 1). Food and water intake returned to baseline between 1 and 2 weeks postoperative for all groups.

The biochemical analysis was done using an analysis of variance with planned comparison (a priori orthogonal comparison) t-tests [29] for lesion and control group comparisons if the ANOVA showed a significant effect. In the frontal cortex there was a significant treatment effect on 5-HT, $F(2,24)=4.54$, $p<0.025$. Planned comparison t-tests demonstrated significant depletions in 5-HT concentrations as compared with control at both doses of 5,7-DHT ipsilateral and contralateral to the side of injection (Table 1). There was a similar pattern of 5-HT decrease in the posterior cortex, $F(2,24)=3.67$, $p<0.05$, with primarily ipsilateral depletions but also some significant bilateral depletions (Table 1). Although we did not analyze the median raphe at the 2 μg dose, there was a significant treatment effect following a 4 μg dose (i.e., control vs. 4 μg), $F(1,18)=6.52$, $p<0.025$. Left lesion produced significant depletions both ipsilaterally and contralaterally while right lesions produced a significant depletion only ipsilaterally (Table 1).

Apart from these depletions in 5-HT concentrations there were no significant depletions of NE in either the frontal cortex, $F(2,24)=0.31$, $p>0.05$, or posterior cortex,

TABLE 1

BIOGENIC AMINE CONCENTRATIONS FOLLOWING CORTICAL INJECTION OF 5,7 DHT

		Concentration ng/mg Wet Tissue Wt. Mean ± SE* (% of Control)					
		Control		Left Lesion Dose of 5,7 DHT		Right Lesion Dose of 5,7 DHT	
Brain Region	Amine Analyzed	(N=8)		2 μg (N=4)	4 μg (N=8)	2 μ (N=4)	4 μg (N=6)
Frontal Cortex	(NE)	422 ± 33	Ipsi	285 ± 49 (68)	376 ± 53 (89)	348 ± 47 (82)	347 ± 52 (82)
			Contra	431 ± 151 (102)	433 ± 61 (103)	449 ± 61 (106)	370 ± 43 (88)
Frontal Cortex	(5-HT)	464 ± 42	Ipsi	284 ± 48† (61)	344 ± 46* (74)	347 ± 69* (75)	326 ± 50* (70)
			Contra	333 ± 51* (72)	333 ± 38* (72)	406 ± 21 (87)	363 ± 62* (78)
Posterior Cortex	(NE)	388 ± 23	Ipsi	260 ± 71 (77)	339 ± 61 (100)	325 ± 59 (96)	296 ± 41 (88)
			Contra	322 ± 78 (95)	307 ± 44 (91)	387 ± 67 (114)	316 ± 48 (93)
Posterior Cortex	(5-HT)	364 ± 38	Ipsi	296 ± 82 (81)	253 ± 43* (69)	238 ± 23† (65)	279 ± 49* (77)
			Contra	282 ± 23* (77)	289 ± 55 (78)	305 ± 20 (84)	243 ± 33† (67)
Caudate Nucleus	(DA)	5078 ± 404	Ipsi	4728 ± 1152 (93)	4284 ± 593 (84)	5060 ± 693 (100)	4313 ± 459 (85)
			Contra	4102 ± 216 (81)	5705 ± 837 (112)	4857 ± 540 (96)	5752 ± 671 (113)
Substantia Nigra	(DA)	543 ± 53	Ipsi	395 ± 88 (73)	549 ± 78 (101)	517 ± 105 (95)	536 ± 68 (99)
			Contra	656 ± 80 (121)	490 ± 121 (90)	643 ± 306 (118)	504 ± 72 (93)
Median Raphe	(5-HT)	1035 ± 93	Ipsi		728 ± 136† (70)		801 ± 195* (77)
			Contra		684 ± 119† (66)		842 ± 195 (81)

*$p<0.05$, †$p<0.025$.
pValues as compared with controls.

$F(2,24)=0.10$, $p>0.05$. Likewise, there were no significant depletions of DA concentrations in either the caudate, $F(2,24)=0.26$, $p>0.05$, or the substantia nigra, $F(2,24)=0.08$, $p>0.05$.

Since statistical analysis showed no significant difference in activity measures between the 2 and 4 μg treatment or control groups, it may be reasonably concluded that we failed to reject the null hypothesis that 5,7-DHT has no effect on activity. We therefore did a power analysis to assess the probability that these results may have missed a significant intergroup difference. Since hyperactivity would be expected to occur between 2 and 4 weeks postoperative [12, 17, 25], we combined the 2 and 4 μg groups so that we could assess the effect of side of injection and determined a mean activity for each animal between days 13 and 28. Mean activity for the 12 right hemisphere injected animals was 99.8±28.1 SD for the 12 left hemisphere injected animals was 100.8±24.7 SD and for 10 control animals was 95.2±19.0 SD. Based on our previous studies in which we demonstrated hyperactivity following cortical lesions, we calculated the mean activity between postoperative days 13 and 28 for suction (36%) [17], cortical undercuts (37%) [11], kainate (39%) [10], 6-OHDA (42%) [26], or DSP-4 (71%) [12] lesions. Thus, we have previously found at least a 36% increase in activity and if we assume that a 36 percent increase in activity would have been detected for right lesion animals and no

difference between left and control, the error variance was 24.8 with 2 and 29 degrees of freedom (i.e, 12 R, 12 L, 8 C) with power 1−B=0.91, for a=0.01 [19]. Thus, the probability was greater than 90% that we would have detected a 36% activity difference given the experimental variance and number of animals.

CONCLUSIONS

This study has demonstrated that unilateral injections of 5,7-DHT with DMI pretreatment produced bilateral depletions of cortical and midbrain serotonin concentrations in rats, but did not affect spontaneous activity.

The most significant implication from this study is that focal destruction of serotonin terminals within the right frontal cortex is not sufficient to produce the lateralized hyperactivity phenomenon and therefore that 5-HT does not appear to be directly involved in asymmetrical behavioral response to cortical lesion. It may also be construed that specific nerve terminals must be destroyed to produce hyperactivity.

The absence of hyperactivity following these serotonin-specific lesions, however, does not mean that 5-HT containing neurons might not play some role in the production of the hyperactivity which follows right-sided ischemic or suction lesions. It may be, for example, that the serotonin depletions produced in the current study were of insufficient magnitude to produce an effect. Most of the serotonin depletions in this study, however, were comparable in magnitude to the catecholamine depletions associated with hyperactivity in previous studies [21,23]. Alternatively, the lack of hyperactivity in 5,7-DHT lesion rats may imply that hyperactivity is the result of a combination or interaction of neurotransmitter depletions (e.g., NE and 5-HT) and that depletion of 5-HT alone is insufficient to produce the effect.

The 5-HT depletions were primarily bilateral in the cortex and raphe. The observed 5-HT depletions, however, failed to demonstrate a clear relationship to either neurotoxin dose, side of injection, or behavioral effect. This lack of dose related effect on 5-HT concentrations might be interpreted as demonstrating a non-specific effect of 5,7-DHT. On the other hand, since our injection delivered a constant volume regardless of dose, toxin spread was presumably the same in both the 2 and 4 μg treated animals and was restricted to a relatively constant area, although presumably much smaller than the analyzed tissue volume since the depletion was only 30 to 40%. Thus, it is possible that we reached a "ceiling effect" by depleting all the 5-HT in the area of neurotoxin diffusion at the 2 μg dose and therefore produced no further depletion at the 4 μg dose. This finding of a lack of dose related effect was found in previous experiments in which we injected other neurotoxins [12,26]. Finally, the bilateral depletions found with 5,7-DHT were also found in certain brain regions using other neurotoxins [12,26]. The mechanism of this bilateral effect is not adequately explained by crossover pathways and at present remains obscure.

To our knowledge, this is the first study of the effects of cortical serotonergic lesions on locomotor activity. Therefore, we cannot directly compare our results with those of other investigators. In addition, in those studies where locomotor activity has been studied in animals with serotonergic lesions in the midbrain, the results have been inconsistent. Electrolytic lesions of the median but not the dorsal raphe have been reported to result in hyperactivity, with associated 30–40% depletions in cortical 5-HT resulting from either type of lesion [8,28]. 5,7-DHT lesions (with protriptyline pretreatment) of the median raphe, however, did not produce any change in locomotor activity despite producing similar cortical depletions [13]. This suggests that the hyperactivity produced by electrolytic lesions may have been due to destruction of non-serotonergic neurons [13]. In another study, lesions were produced in the ascending serotonergic pathways with 5,7-DHT and protriptyline. Destruction of either medial or lateral pathways did not produce any changes in activity, while destruction of both pathways produced decreased activity [7]. The current study would support the view that cortical serotonergic pathways are not directly involved in spontaneous hyperactivity.

There is an increasing amount of evidence that the hemispheres of the rat brain are functionally [2, 3, 5], anatomically [4,9] and neurochemically [5, 17, 25, 27] asymmetrical. Our current results suggest that the asymmetrical behavioral response to brain injury in the rat may be neurotransmitter specific. This relative specificity of neurotoxic lesions on spontaneous activity may provide additional information about the mechanisms and behavioral significance of these asymmetries in the rat brain.

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