Prolonged hyperreactivity of septal rats with intraventricular pretreatment of 6-hydroxydopamine

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Fifty-four male rats were divided into four groups according to the pretreatment of 6-hydroxydopamine ($250 \mu\text{g} \times 2$) and septal lesions. The magnitude of reactivity to sensory stimulation was estimated by a 4-point rating scale. The rats that received septal lesions without 6-hydroxydopamine showed a marked increase in reactivity on the first day after surgery but a day-by-day decline in their hyperreactivity. On the other hand, the increased reactivity in 6-hydroxydopamine-pretreated septal rats was maintained over the entire course of the testing period, 130 days. Brain monoamine concentrations were assayed 20 weeks after the first injection of 6-hydroxydopamine. Norepinephrine in whole brain was depleted to 13.1% of control level, dopamine decreased to 57.9% of control, but 5-hydroxytryptamine was not affected. The results indicate that long-lasting brain catecholamine depletion prolongs septal hyperreactivity in the rat.

The hypothalamus, in particular the ventromedial subdivision, plays a crucial role in mediating some emotional behaviors, and the amygdala seems to modulate hypothalamic activities. But the function of the septum, which is anatomically located between the hypothalamus and the amygdala, is not yet fully understood with regard to emotional behavior.

The involvement of the septum in emotional behavior was first demonstrated by Fulton and Ingraham (1929), who reported an increase in emotional reactivity following bilateral septal lesions in rats. Their results showed an increase in flight or attack behavior in response to formerly neutral stimuli immediately following the destruction of septal areas. Characteristic of this septal hyperreactivity are the facts that its magnitude diminishes with time and the rate of decrease in magnitude is significantly influenced by the handling of the treated animal (Brady & Nauta, 1953, 1955; Reynolds, 1965). With daily handling, septal hyperreactivity subsided within a week, whereas with infrequent handling rats required more than 30 days to return to a normal state of reactivity. However, it is not clear what the effect of this handling is at the neurochemical level.

Bernard, Berchek, and Yutzey (1975) reported significant reductions in norepinephrine (NE) and dopamine (DA) levels in whole brain 48 h after septal lesions. But over a longer time course, Montgomery and Christian (1973) found near preoperative NE levels in whole brain 6 days after septal lesions. These findings indicate a time dependence of the NE con-

centration roughly correlated with that of septal hyperreactivity.

If catecholamine (CA) is implicated in the hyperreactivity of septal rats, chronic depletion of CA prior to lesioning should delay recovery. It is well known that, through selective terminal destruction, intraventricular injection of 6-hydroxydopamine (6-OHDA) in adult animals chronically depletes CA levels in various parts of the brain (Breese & Trayler, 1970; Uretsky & Iversen, 1970). It might be expected that pretreatment of septal rats with 6-OHDA would prolong septal hyperreactivity by keeping catecholamine concentrations artificially low. The present study was carried out in order to determine whether this was the case.

METHOD

Animals

Fifty-four male Wistar rats, weighing 250-300 g, were used. The animals were housed individually and permitted free access to food and water throughout the experiment.

A cannula (26-ga stainless steel tube) was implanted under stereotaxic guidance in the lateral ventricle while the animal was anesthetized with sodium pentobarbital (50 mg/kg, ip). After 3 days of recovery from surgery, each animal was given two 20- μl doses of .9% saline vehicle solution in succession, 24 h apart. The volume and concentration of injected solution was determined by the results of Breese and Trayler (1970) and Uretsky and Iversen (1970). Of the animals injected, 32 received 250 μg of 6-OHDA in the vehicle solution into the lateral ventricle and 22 received vehicle only. Thirty minutes prior to the first 6-OHDA injection, each animal received pargyline (50 mg/kg, ip). No treatment was given prior to the second 6-OHDA injection.

Electrolytic Injury of Septal Area

Ten days after the first injection of 6-OHDA or the vehicle solution, 31 rats (18 of the 32 6-OHDA treated rats and 13 of the 22 vehicle treated rats) received septal surgery under stereotaxic

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guidance during pentobarbital anesthesia (50 mg/kg, ip). A bipolar electrode made of two twisted stainless steel wires, each 200 μ m in diameter, were positioned bilaterally at points 1.0 mm anterior to bregma, .5 mm lateral to midline of the skull, and 5.0 mm below dura. Direct current (about 3.0 mA) was passed for 45 sec. The remaining 23 rats underwent the same treatment, except that the electrodes were lowered to 3.0 mm below dura and no current was passed.

The animals were divided into four groups according to drug and surgical treatment. Group 6-OHDA + S received two injections of 6-OHDA and then septal lesions; Group 6-OHDA + Sham received two injections of 6-OHDA followed by a sham operation; Group V + S was injected with two intraventricular vehicle solutions followed by bilateral septal lesions; and Group V + Sham received two vehicle solutions and the sham operation.

Behavioral Test

To evaluate changes in reactivity associated with 6-OHDA treatment and septal damage, a rating technique adapted from the method of Brady and Nauta (1953) was used. Ratings of animal reactivity were made for the following four behavioral components: (a) reaction to a forceps brought close to the snout, (b) reaction to a slight tapping on the back with the forceps, (c) resistance to capture with a gloved hand, (d) amount of vocalization during the above three tests. A 4-point rating scale was used for each of the behavioral components. A 0 rating was assigned to a rat that remained calm during testing, and a rating of 3 was assigned to a rat that exhibited a defensive, upright posture in response to the forceps (a), a jumping or flight response to the tapping (b), biting or severe resistance to capture (c), and incessant vocalization during the testing (d).

Except for the day of operation when the septal lesions were made, behavioral testing was performed every day, from 3 days before injection of 6-OHDA or vehicle solution to the day when the rat was sacrificed.

Scores obtained from the four behavioral components were summed to provide a hyperreactivity index, and analyzed statistically by the Student *t* test.

Chemical Assay

Twenty weeks after the first intraventricular injection of 6-OHDA or vehicle solution, the animals in Groups 6-OHDA + S and V + Sham were decapitated. The brains were quickly removed from the calvarium, cut into three pieces: hypothalamus including ventral part of thalamus, amygdala and piriform cortex, and the residual part of the brain excluding cerebellum. Then the sections were frozen on dry ice and weighed. It took, at most, 3 min from the time the animals were sacrificed until the time the tissues were frozen. These tissue samples were stored at -30°C until assay, which always took place within 1 week. The concentrations of three monoamines in each of the brain sections were determined by the method of Karasawa et al. (1975).

RESULTS

Behavioral Changes After 6-OHDA Alone

The first treatment of 6-OHDA strongly inhibited spontaneous motor activity in the rats. All of the treated rats crouched in a dark corner of their home cages for 2 to 5 h after injection. Some of the animals jumped abruptly and squeaked loudly several times. On the next day, when the second 6-OHDA was given, it was not possible to discriminate between drug- and vehicle-treated rats in their spontaneous behavior. The second 6-OHDA revealed no acute effect on behavior in any of the animals.

There were no significant differences in rating scores between the two groups for 10 days until septal surgery.

Hyperreactivity Caused by Septal Lesions in 6-OHDA Pretreated Rats

Figure 1 shows the mean rating scores in all four groups on pre- and postoperative days. A marked increase in rating scores was obtained in two septal lesioned groups, 6-OHDA + S and V + S. On the first 2 days after septal surgery, all rats in both groups showed a marked change in reactivity in response to the extended forceps. These rats took a defensive upright posture, vocalized strongly, or demonstrated attack behavior, grasping the forceps with their forelimbs and biting it when it was brought close to the snout. Most rats jumped explosively when their backs were tapped lightly by the forceps. Additionally, the rats in Group 6-OHDA + S showed a kind of cataleptic syndrome of behavior. Once they took the upright posture in response to the presented forceps, they kept the same posture for at least 1 min, even after the stimulus was removed. This characteristic feature of response was not observed among any of the rats in Group V + S.

The hyperreactivity of animals in Group 6-OHDA + S persisted over the entire course of testing. No significant differences were found between daily postoperative scores within Group 6-OHDA + S. On the other hand, the hyperreactivity in Group V + S was gradually attenuated. Although there were significant differences in rating scores between Group V + S and Group V + Sham by the fifth postoperative day, from the sixth day on, no significant differences were found in rating scores between these two groups.

Figure 1. Mean rating score ($\pm$ S.E.M.) of reactivity on pre- and postoperative days in four groups. Filled circles are Group 6-OHDA + S, open circles are Group V + S, filled triangles are Group 6-OHDA + Sham, and open triangles are Group V + Sham. Animals in Groups 6-OHDA + S and 6-OHDA + Sham were administered 6-OHDA (250 μ g $\times$ 2) prior to the surgical operation. **p* < .05, ***p* < .01 vs. V + Sham group by *t* test.

Some rats in Group 6-OHDA + Sham appeared to show a slight increase in reactivity to tactile stimulation. However, no significant differences were found in rating scores between 6-OHDA + Sham and V + Sham over the postoperative testing days.

Brain Monoamine Levels in Groups 6-OHDA + S and V + Sham

Table 1 shows the results of brain monoamine concentrations 20 weeks after 6-OHDA. A significant depletion of NE and DA in whole brain was seen in the animals in Group 6-OHDA + S in comparison with the concentrations of CA in the animals in the control group, V + Sham. NE level in whole brain was depleted to 13.1% of control level, and DA decreased to 57.9% of the control level. The concentration of whole-brain 5-hydroxytryptamine (5-HT), on the other hand, was not different between the two groups.

As to the results of discrete brain areas, NE depletion in amygdala and diencephalon was profound compared with DA depletion in these areas. No differences were found in 5-HT level in the three brain areas studied.

DISCUSSION

The major finding obtained in the present study was that the pretreatment of 6-OHDA prolonged the hyperreactivity induced by bilateral septal lesions in the rat.

Septal rats without 6-OHDA showed a marked increase in reactivity to visual and tactile stimulations from the first day after surgery, and they gradually lost their hyperreactivity to the stimulations with repeated daily testing. There were no statistically significant differences between the mean rating scores of the septal group and those of the sham-operated group (V + Sham) after the fifth postoperative day in this experiment. Septal rats with 6-OHDA, on the other hand, continued to show high reactivity scores throughout the 130 test days.

In this study, any behavioral changes in rats were not found with 6-OHDA treatment per se. This find-

ing agrees with the results of other studies (Burkard, Jalfre, & Blum, 1969; Evetts, Uretsky, Iversen, & Iversen, 1970; Laverty & Tayler, 1970). Nakamura and Thoenen (1972), however, observed hyperirritability after intraventricular injections of 6-OHDA in rats. Coscina, Seggie, Godse, and Stancer (1973) also reported that rage reaction was seen with single intracisternal 6-OHDA treatment. The rage reaction was maximal on the ninth day after injection and gradually diminished with daily handling. These results do not agree with ours. In order to examine the reproducibility of the results obtained in this experiment, 6-OHDA was given to another group of seven naive rats, and the result was the same—no detectable changes were found in these 6-OHDA treated rats. It is not clear why the discrepancy occurred. It should be pointed out, however, that in the daily behavioral testing in this study, we first brought the forceps close to the snout of the rat and then gave the tactile stimulation, tapping the back of the animal slightly with the forceps, since the septal rat had been known to be sensitive not only to tactile stimulation but also to visual stimulation (Brady & Nauta, 1953; Fried, 1973). Coscina et al. (1973) and Nakamura and Thoenen (1972) did not employ visual threats in their behavioral testing.

The nuclei of the septum send efferent fibers to the vasolateral portion of the amygdala (Nauta, 1958; Powell, 1963; Swanson & Cowan, 1979). Electrical stimulation of the septum (Siegel & Skog, 1970) and the amygdala (Egger & Flynn, 1963) increases the latencies of hypothalamically induced attack behavior in the cat. These results suggest that the septum has a regulating function on aggressive behavior.

The results of monoamine concentration assayed 20 weeks after the first injection of 6-OHDA showed that NE and DA levels significantly decreased in the whole brain as well as in diencephalon and amygdala. 5-HT was not affected in all discrete brain areas assayed. The septal lesions per se decreased NE and DA levels in the brain 48 h after surgery (Bernard et al., 1975), but this effect was not observed 7 days after surgery (Montgomery & Christian, 1973). NE

Table 1
Effects of 6-OHDA (250 Micrograms $\times$ 2) on Brain Monoamine Concentrations

Brain Area	Brain Monoamine Concentrations (in Nanograms per Gram of Tissue)														
	Control "V + Sham"						"6-OHDA + S"								
	NE		DA		5-HT		NE			DA			5-HT		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	PC	Mean	SEM	PC	Mean	SEM	PC
Diencephalon	982	14	505	7	833	103	193	35	19.7	162	10	32.1	757	41	90.9
Amygdala	419	33	713	124	636	58	60	17	14.3	479	43	67.2	620	57	97.5
Residual Parts of the Brain	390	34	1045	52	488	30	41	7	10.5	631	30	60.4	476	56	97.5
Whole Brain	472	104	935	46	542	31	62	7	13.1	541	58	57.9	575	62	106.1

Note—Brain monoamines were assayed 20 weeks after the first intraventricular injection of 6-OHDA. PC = percent of controls. "6-OHDA + S" animals received septal injuries 10 days after the first dose of 6-OHDA.

and DA are supposed to be inhibitory synaptic transmitters in the brain (Szabadi, 1979). The depletion of these transmitter substances in diencephalon and amygdala is thought to elicit the disruption of septo-amygdaloid influences on the hypothalamus. Fried (1973) postulated that septal hyperreactivity is induced by dysfunction in the septoamygdalo-hypothalamic neural circuit.

Gage and Olton (1976) reported that L-dopa, a metabolic precursor of catecholamines, reduced septal hyperreactivity. Marotta, Logan, Potegal, Glusman, and Gardner (1977) obtained similar effects with dopaminergic agonists, apomorphine, peribedil, and amphetamine. The results obtained by these recent studies are not consistent with the effects of chlorpromazine and haloperidol. These dopaminergic blockers have a transient depressant action on the septal rage syndrome (Raitt, Nelson, & Tye, 1961; Stark & Henderson, 1966). Marotta et al. (1977) mentioned that the effects of these agents resembled the effects exerted by such nonspecific sedatives as methohexital. They concluded that the septal syndrome was concerned with dysfunction of the dopaminergic system, and that dopaminergic agents facilitated recovery from the septal syndrome by diaschistic processes in the dopamine-rich mesolimbic system. It is conceivable that long-lasting CA depletion by 6-OHDA in this study might prevent these processes. In the biochemical assay, it was found that 6-OHDA lowered both NE and DA concentrations in the brain areas, although DA was depleted to a lesser extent than NE. Which system, NE or DA, is more crucially involved in septal hyperreactivity has to be determined in the next step.

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