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Reinforcing Properties of Cocaine in the Medial Prefrontal Cortex: Primary Action on Presynaptic Dopaminergic Terminals

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GOEDERS, N. E. AND J. E. SMITH. *Reinforcing properties of cocaine in the medial prefrontal cortex: Primary action on presynaptic dopaminergic terminals.* PHARMACOL BIOCHEM BEHAV 25(1) 191–199, 1986.—The presynaptic mechanisms involved in the initiation of cocaine reinforcement were investigated using neurotoxin lesions. Rats were trained to intracranially self-administer cocaine (50 to 90 pmol) into the medial prefrontal cortex and after dose-effect analyses were completed, each rat received a unilateral 6-hydroxydopamine lesion (4 μg in 0.2 μl) at the self-administration site. The lesion selectively decreased dopamine content in the medial prefrontal cortex (−45%) and decreased cocaine-maintained responding to vehicle levels. Lever-pressing could be reinstated by substituting dopamine (300 pmol) but not serotonin for cocaine. Dopamine self-administration was attenuataed by including equimolar concentrations of the D_2 dopaminergic antagonist sulpiride in the injectate. These results suggest that the initiation of reinforcing neuronal activity in the medial prefrontal cortex appears to result in part through the direct interaction of cocaine with presynaptic reuptake sites associated with dopaminergic nerve endings. The resulting increased synaptic concentration of the neurotransmitter may then interact with postsynaptic D_2 binding sites to activate neuronal systems involved in the mediation of this reinforcement.

Cocaine reinforcement Intracranial self-administration 6-Hydroxydopamine Medial prefrontal cortex Dopamine

THE non-medical use of cocaine has rapidly increased during the last decade. Behavioral studies have shown that the response-contingent administration of the drug will maintain long and complex sequences of behavior by animals and humans under various schedules of reinforcement [14, 15, 23]. Self-administration is thought to be maintained by the reinforcing neuronal activity that results following the interaction of the drug with specific receptors. The neurobiological mechanisms involved in these complex processes are under investigation, but have yet to be conclusively elucidated. Although the effects of cocaine on neurotransmission are complex, the primary neurochemical action appears to be an inhibition of biogenic amine neurotransmitter uptake into presynaptic nerve endings. The drug decreases the reuptake of norepinephrine [9, 24, 42], dopamine [25, 29, 32] and serotonin [44,46] without affecting release except at high concentrations [7,43]. Brain dopamine and acetylcholine turnover rates are increased by cocaine [4,34] while norepinephrine and serotonin turnover are decreased [2,16]. Although cocaine appears to affect several neuronal systems, animal intravenous self-administration experiments suggest an important role for dopamine in the neuronal processes related to its reinforcing properties. Cocaine-maintained responding by rats and rhesus monkeys is increased in a dose-related manner after systemic administration of the dopaminergic receptor antagonists pimozide [8], haloperidol [6], alpha-flupenthixol [13] and sulpiride [40], suggesting an attenuation of reinforcing efficacy. 6-Hydroxydopamine lesions of the nucleus accumbens disrupt cocaine self-administration if sufficient dopamine loss is achieved [37,38]. Similar lesions of the ventral tegmental area (which contains the cell bodies for the mesolimbic/mesocortical dopaminergic pathway) also modify cocaine self-administration, but the degree of attenuation does not correlate with decreases in dopamine content in the nucleus accumbens [39]. These data suggest that dopaminergic innervations of other structures

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(i.e., medial prefrontal cortex or olfactory tubercle) may participate in the neuronal processes mediating reinforcement following the response-contingent presentation of cocaine.

Specific cocaine binding has been demonstrated in the mouse [35, 36, 41] and rat brain [26,28] using homogenate binding assays. These binding sites are proposed to be localized on presynaptic nerve terminals associated with dopaminergic [28] or serotonergic [35] reuptake sites. Preliminary data obtained using light microscopic quantitative autoradiography have also identified a heterogeneous distribution of specific cocaine binding in the rat brain that includes high densities in the medial prefrontal cortex [22]. The recent report of the direct self-administration of cocaine into the medial prefrontal cortex but not the nucleus accumbens or ventral tegmental area [17] suggests that a discrete population of these binding sites may be involved in the initiation of reinforcing neuronal activity that follows the response-contingent presentation of the drug. The co-infusion of equimolar concentrations of the D_2 dopaminergic receptor antagonist sulpiride attenuated intracranial cocaine self-administration and produced patterns of responding consistent with decreasing the cocaine dose, while D_1 dopaminergic, muscarinic-cholinergic and beta-noradrenergic receptor antagonists either did not modulate drug-intake or had minimal effects [20]. These data suggest that the initiation of cocaine reinforcement processes results in part from direct or indirect interactions with D_2 receptors in the medial prefrontal cortex. This investigation was designed to further elucidate the neuronal mechanisms involved by determining if the reinforcement-relevant receptors are localized on presynaptic dopaminergic terminals or on postsynaptic neurons by lesioning the self-administration site with 6-hydroxydopamine.

METHOD

Subjects

Four experimentally naive male Fischer 344 rats 90 to 150 days old at the beginning of this experiment were used. Each animal was housed in an individual cage on a reversed 12-hour light-dark cycle (light onset at 20:00 hr) with free access to food (Purina Rat Chow) and water.

Surgery

Sodium pentobarbital anesthesia (50 mg/kg, IP) with methylatropine sulfate pretreatment (10 mg/kg, IP) was used during surgery. The animals were stereotactically implanted with unilateral 22-gauge guide cannulae (Plastic Products Co.) into the right medial prefrontal cortex (10.05 mm anterior to lambda, 0.6 mm from the midline, 2.1 mm ventral to dura [30]) using a modification of a previously described procedure [33]. Briefly, appropriately placed holes were drilled in the calvarium exposing the dura mater. The guide cannulae were inserted 2.1 mm below the surface of the brain into the medial prefrontal cortex and secured to the skull using dental acrylic and 00 stainless steel self-tapping screws. A 28-gauge dummy cannula (Plastic Products Co.), extending 0.5 mm beyond the tip of the guide was inserted to prevent the formation of obstructions and remained in place except during testing. The animals were injected with sterile penicillin G procaine suspension (75,000 units, IM) and allowed a minimum of two weeks to recover from surgery.

Apparatus

Rectangular, Plexiglas operant-conditioning chambers (29×27×30 cm) contained in ventilated, sound-attenuating enclosures were used for the behavioral sessions. A red stimulus light located above a lever on one wall of the experimental chamber indicated the availability of response-dependent microinfusions. Following the successful completion of the response requirement, the red light was extinguished, a white light illuminated and a tone presented concurrent with the delivery of 100 nanoliters of drug solution infused over 5 seconds. Each microinfusion was followed by a 30-second time-out with the stimulus lights darkened and responses by the animal monitored but having no scheduled consequences. Experimental sessions were controlled by a Rockwell Aim 65 Microcomputer with a Microcomputer Control System (Minneapolis, MN).

Intracranial microinfusions were delivered by an adaptation [17,18] of the electrolytic microinfusion system [1,5], which was mounted directly onto the guide cannula (Plastic Products, Roanoke, VA). Microinjections were produced by passing a direct current between a silver anode and platinum cathode contained within an air-tight drug reservoir, with the resulting evolution of hydrogen gas forcing a reproducible amount of drug solution out through a 28-gauge injection cannula extending 0.5 mm beyond the tip of the guide. The amount of solution injected was directly proportional to the volume of hydrogen evolved, which was proportional to the current intensity and duration. This system was calibrated to deliver consistent 100±7 nanoliter volumes over 5 seconds with a 200 μA injection current [19]. A small quiescent current (6 μA) prevented the redissolution of hydrogen evolved during the experimental session. A flexible spring-covered lead connected the microinjection system to a two-channel mercury commutator (Mercotac, Inc.) and a counter-balance to permit relatively unrestrained movement of the animal during testing.

Pre-Lesion Behavioral Procedures

The rats were tested for intracranial self-administration every third day during 8-hour sessions beginning at the start of their active cycles (08:00 to 09:00 hr). A maximum of 40 microinjections was allowed during each experimental session to minimize potential cytotoxic effects. Dose-response curves were individually determined for each animal using a previously described procedure [20]. Briefly, the rats were initially tested on a continuous schedule of reinforcement during which each lever-press resulted in a microinfusion of 50 pmol of cocaine HCl (Mallinckrodt) dissolved in 100 nanoliters of artificial cerebrospinal fluid (CSF). The ionic composition of the vehicle, expressed as grams/liter, was as follows: NaCl, 8.10; KCl, 0.25; $CaCl_2$, 0.14; $MgCl_2$, 0.11; $NaHCO_3$, 1.76; NaH_2PO_4, 0.07; urea, 0.13; glucose, 0.61; pH 5.7. When lever-pressing stabilized for three consecutive sessions at this dose, the concentration was increased or decreased by 5 to 25 pmol. The magnitude of each dose manipulation was independently determined for each animal by comparing the effects of previous changes in concentration on self-administration. If the behavior was sensitive, then the dose was modified by only 5 pmol. If rates of self-administration were less affected, then larger changes were made (15 to 25 pmol). This procedure was repeated with a minimum of three sessions between each dose change until drug-intake decreased to vehicle levels at both the higher and lower concentrations.

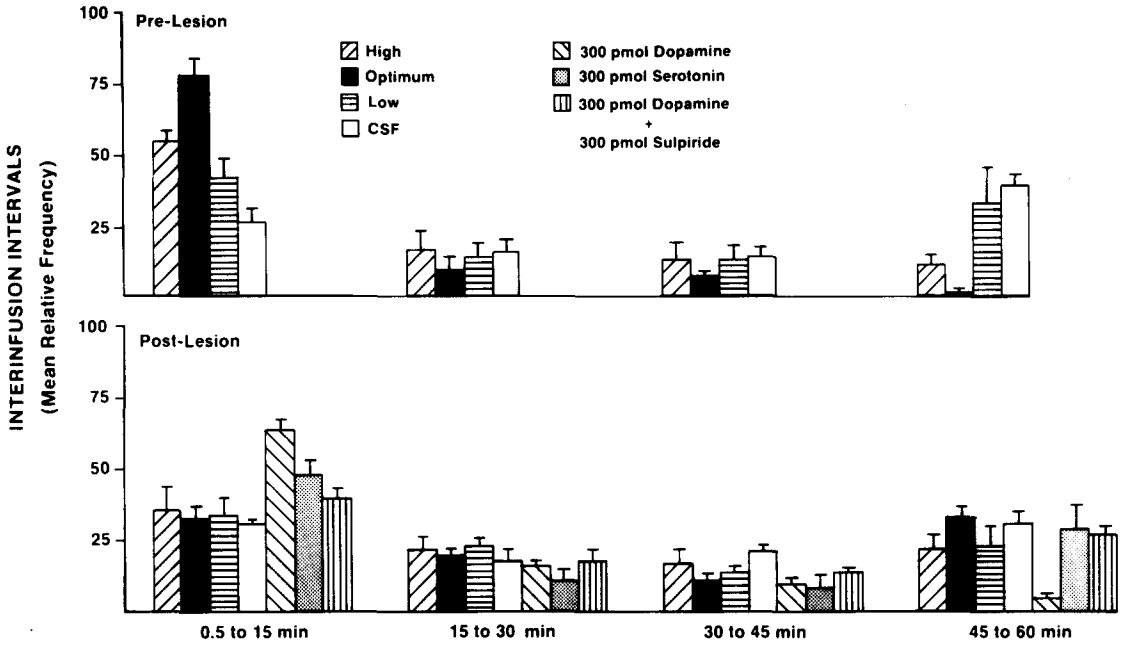

FIG. 1. Mean relative frequency of interinfusion intervals for intracranial self-administration of cocaine into the medial prefrontal cortex of four rats before (upper panel) and after (lower panel) 6-hydroxydopamine lesions at the self-administration site. Optimum represents the concentration of cocaine (50–90 pmol) that resulted in maximum rates and consistent drug-intake, while low and high represent the lowest (10–25 pmol) and highest (75–150 pmol) concentrations of the drug, respectively, that maintained responding in each animal. Values are the mean percentage of total interinfusion intervals that occurred during each 15 minute division (±standard error of the mean) for three days of stable responding (variations <10%) at each dose for each of the four animals. Upper panel: Although the relative frequencies for vehicle and the high and low doses of cocaine were not significantly different in the second and third divisions, the significance of the differences between doses and vehicle was evident in the first and fourth division (Table 1). In the first division, all comparisons among the relative frequency distributions were significantly different ($p<0.05$), while in the fourth division, all comparisons except low vs. CSF and optimum vs. high were significantly different ($p<0.05$). The patterns of self-administration are clearly different for CSF and these concentrations of cocaine. Lower panel: The 6-hydroxydopamine lesion flattened the dose-response curve (Fig. 2) and resulted in patterns of responding that were not significantly different from vehicle ($p>0.05$) prior to the lesion at any concentration tested. The substitution of dopamine (but not serotonin) reinstated self-administration with the rates and patterns of drug-intake not significantly different ($p>0.05$) from the optimum or high dose of cocaine prior to the lesion (Table 2). The co-infusion of sulpiride with dopamine decreased drug-intake with rates and patterns of responding similar to vehicle or a low dose of cocaine. Dopamine self-administration in these 6-hydroxydopamine lesioned animals is clearly similar to that resulting from the optimum dose of cocaine prior to the lesion.

The parameters of self-administration monitored included the mean number of infusions delivered each session and the mean relative frequency of the interinfusion intervals. The relative frequency of the interinfusion intervals is a reliable empirical measure of the pattern of self-administration [20]. The interinfusion intervals (duration between successive microinjections) were counted separately in four 15-minute divisions (0–15 minutes; 15–30 minutes; 30–45 minutes; 45–60 minutes) and a frequency distribution was generated for each session. Relative frequency was calculated by dividing the number of infusions in each 15-minute division by the total number of infusions and multiplying by 100. These parameters were used to delineate changes in behavior resulting from changes in drug dose or the lesion.

Lesions

When dose effect analyses were completed and stable baselines of drug-intake were again obtained, each animal received a unilateral 6-hydroxydopamine lesion of the medial prefrontal cortex at the self-administration site. The rats were pretreated with desmethylimipramine (25 mg/kg, IP) 35 minutes prior to the delivery of the anesthetic (sodium pentobarbital, 25 mg/kg, IP; methylatropine sulfate, 5 mg/kg, IP) to inhibit the uptake of the neurotoxin into noradrenergic neurons. Forty-five minutes following the desmethylimipramine injection, the dummy cannula was removed and a 28-gauge injection cannula inserted into the self-administration guide. This cannula was connected by a flexible polyvinylchloride tubing (0.76 mm o.d. × 0.25 mm i.d.) to a 1.0 μl microsyringe (Hamilton) attached to a motor-driven syringe pump. Each rat received 4 μg of 6-hydroxydopamine (Sigma) dissolved in 0.2 μl of isotonic saline (with 0.02% ascorbic acid added to prevent autooxidation) delivered through the injection cannula over 6 minutes, with the cannula left in place for an additional 10 minutes to prevent the diffusion of the neurotoxin into the guide. Normal drug flow was confirmed before and after the microinfusion, the dummy cannula was replaced and the animals were returned to their home cages. The neurotoxin was de-

TABLE 1

STATISTICAL ANALYSIS OF THE EFFECTS OF COCAINE DOSE ON THE MEAN RELATIVE FREQUENCIES OF THE INTERINFUSION INTERVALS FOR INTRACRANIAL SELF-ADMINISTRATION INTO THE MEDIAL PREFRONTAL CORTEX

Interval	ANOVA[1] F(3,16)	Cocaine Dose	Comparison[2] Cocaine Dose High	Low	Optimum
1		CSF	9.03	4.94	16.10
(0–15 min)	185.43†	Optimum	−7.07	−11.16	—
		Low	4.09	—	—
2		CSF	0.41*	−0.93*	−3.37
(15–30 min)	11.43†	Optimum	3.77	2.44*	—
		Low	1.33*	—	—
3		CSF	−0.48*	−0.87*	−4.67
(30–45 min)	18.28†	Optimum	4.19	3.80	—
		Low	0.40*	—	—
4		CSF	−6.00	1.33*	−8.23
(45–60 min)	59.99†	Optimum	2.23*	6.90	—
		Low	−4.67	—	—

Values obtained from a [1]completely randomized 2-factor analysis of variance [10] followed by [2]Tukey's all pairwise comparisons among treatment means [27] for N = 4.

*Non-significant comparisons at $p<0.05$ ($t = 2.86$).

†$p<0.005$.

livered on a day normally scheduled for self-administration (i.e., three days after the last session and three days before the next). Post-lesion self-administration sessions were initiated three days later, and the rates of drug-intake were compared with those obtained pre-lesion.

Post-Lesion Behavioral Procedures

Following the lesion, the rats were tested for self-administration with the optimum dose (50–90 pmol) of cocaine (that dose resulting in the highest stable rate of self-administration) for three behavioral sessions with the rates and patterns of drug-intake monitored. Then, each rat was exposed to duplicate sessions of the highest (75–150 pmol) and lowest (10–25 pmol) doses that maintained responding (determined for each in the pre-lesion dose-response investigations) and vehicle, followed by another probe at the optimum dose. The rates and patterns of intake at each concentration were compared to pre-lesion.

Neurotransmitter Substitution

Self-administration of dopamine was then assessed to determine if postsynaptic receptor-mediated processes were still viable. Initially, doses of 250 to 500 pmol of dopamine (Sigma) dissolved in artificial cerebrospinal fluid were used (approximately 3 to 5 times the optimum dose of cocaine), and the concentration maintaining maximum rates of lever-pressing determined for each rat. When responding maintained by dopamine stabilized, the optimum dose of cocaine was again tested. Doses of serotonin (Sigma) equimolar to dopamine were then assessed to determine if the self-administration of dopamine post-lesion was specific since the medial prefrontal cortex also receives serotonergic input that could potentially be damaged by 6-OHDA [12]. Finally, equimolar concentrations of sulpiride (Delagrange) were included with dopamine to determine if this self-administration was mediated through dopaminergic D_2 receptors post-lesion as cocaine was prior to the lesion [20].

Neurochemical Procedures

Following the completion of the testing regimen, the animals were sacrificed by total immersion in liquid nitrogen until frozen (5 minutes), and the heads were removed and stored at −70°C. The heads were warmed to −18°C in a cryostat (Damon/IEC Division), the brains were removed and a cut was made in the coronal plane along the cannulae tract. Guide cannulae locations were verified and recorded, and the right (self-administration site) and left medial prefrontal cortices and right and left nucleus accumbens were separately microdissected from 500 μm serial sections using a stereomicroscope. The tissue samples were individually pulverized in liquid nitrogen with a stainless steel mortar and pestle and stored at −70°C until assay.

The content of norepinephrine (NE), dopamine (DA), serotonin (5-HT), tyrosine (Tyr), and 3,4-dihydroxyphenylacetic acid (DOPAC) were concurrently measured in each tissue sample using a previously reported procedure [3]. Briefly, these compounds were extracted from tissue with 20 volumes of 4°C 1 N formic acid/acetone (v/v:15/85), and 100 pmol of 3,4-dihydroxybenzyl-amine (DHBA) were added to each sample to correct for recovery. The tissue homogenates were centrifuged at 2000 × g for 10 minutes at 4°C, the supernatant collected and the tissue pellets saved for protein determination [31]. Three volumes of heptane/chloroform (v/v:8/1) were added to the supernatant, mixed, centrifuged and the organic layer and lipid interphase discarded. The aqueous layer was taken to dryness under a stream of nitrogen and stored at −20°C until analysis. The extracts were assayed with high pressure liquid chromatography and electrochemical detection using a reverse-phase 10 micron μBondapak C_{18} column (3.9 mm × 30 cm; Waters Associates, Inc.) and a 0.1 M citrate-phosphate buffer mobile phase, pH 3.5, containing 0.01% sodium octyl sulfate (Eastman) and 5% methanol. Contents were determined by a direct comparison of the peak height with external standards and the values corrected for recovery and expressed as pmol/mg protein.

Statistical Procedures

The significance of differences in the relative frequency distributions of interinfusion intervals was evaluated with a two-factor randomized analysis of variance [10], followed by Tukey's all pairwise comparisons among treatment means [27].

RESULTS

All four rats intracranially self-administered cocaine, with six behavioral sessions generally required to establish stable rates of self-administration. An average of 62 sessions (31 weeks) were necessary to complete the pre-lesion dose-response analyses. An additional fifteen sessions (eight weeks) were required for the remaining animals to complete the post-lesion experimental manipulations. One rat dislodged the guide cannula before the end of the post-lesion neurotransmitter substitutions.

Cocaine self-administration into the medial prefrontal cortex was dose-related with maximal rates of responding (38–40 infusions/session) obtained with 50 to 90 pmol/infu-

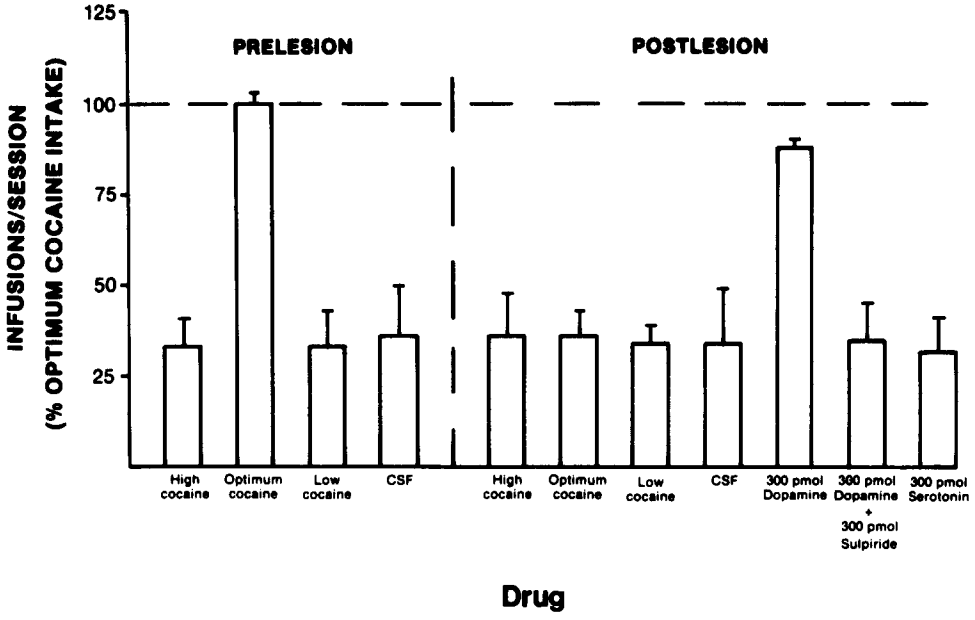

FIG. 2. Effects of 6-OHDA lesions of the medial prefrontal cortex on the intracranial self-administration of cocaine or biogenic amine neurotransmitters into the same brain region. Optimum represents the cocaine dose (50–90 pmol) that resulted in maximum rates of responding (38–40 infusions/session), while low and high represent the lowest (10–25 pmol) and highest (75–150 pmol) concentrations of the drug that maintained responding in each rat. Values are means (±standard error of the mean) for double determinations in four rats (except dopamine plus sulpiride and serotonin where N=3) and are expressed as the percent of baseline drug-intake maintained by the optimum dose (100%) before the lesion. The lesion decreased drug-intake with the optimum dose to vehicle levels, but responding was reinitiated with 300 pmol dopamine.

TABLE 2

STATISTICAL ANALYSIS OF THE EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE MEDIAL PREFRONTAL CORTEX ON THE MEAN RELATIVE FREQUENCIES OF THE INTERINFUSION INTERVALS FOR INTRACRANIAL SELF-ADMINISTRATION INTO THE MEDIAL PREFRONTAL CORTEX

Interval	ANOVA[1] F(5,24)	Post-Lesion Microinjection	Comparison[2] Cocaine Dose (pre-lesion) High	Low	Optimum	CSF
1 (0–15 min)	191.63†	Cocaine (Optimum)	9.56	3.93	19.29	−2.86*
		Dopamine 300 pmol	−4.25	−9.88	5.47	−16.68
4 (45–60 min)	75.67†	Cocaine (Optimum)	−7.03	−0.12*	−10.33	1.85*
		Dopamine 300 pmol	2.21*	9.12	−1.09*	11.09

Values obtained from a [1]completely randomized 2-factor analysis of variance [10] followed by [2]Tukey's all pairwise comparisons among treatment means [27] for N = 3.

*Non-significant comparisons at $p<0.05$ ($t=3.09$).

†$p<0.005$.

TABLE 3

CONTENT OF DOPAMINE, DIHYDROXYPHENYLACETIC ACID, SEROTONIN, NOREPINEPHRINE, AND TYROSINE IN RIGHT AND LEFT MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS OF RATS INTRACRANIALLY SELF-ADMINISTERING COCAINE AND RECEIVING 6-OHDA LESIONS OF THE RIGHT MEDIAL PREFRONTAL CORTEX

		DA	DOPAC	5-HT	NE	TYR
Medial Prefrontal Cortex	Right	10.7 ± 1.7*	3.8 ± 0.7*	28.6 ± 4.9*	19.7 ± 2.7	852 ± 245
	Left	19.6 ± 2.0	5.7 ± 2.2	53.0 ± 5.7	19.1 ± 1.9	672 ± 152
Nucleus Accumbens	Right	352.5 ± 32.9	34.5 ± 8.0	70.8 ± 12.8	24.3 ± 3.4	
	Left	389.1 ± 37.7	32.5 ± 1.7	68.9 ± 13.9	30.0 ± 3.3	

Values are means ± standard deviations for N = 4.
The significance of the differences determined with Student's *t*-tests were: $*p<0.01$.

sion which is consistent with previous reports [17,20]. Significant changes in drug-intake often occurred after increasing or decreasing the concentration by 5 or 10 pmol. Even though the rates of self-administration declined similarly as the dose was either increased or decreased from optimum levels, the mean relative frequency measurements of the interinfusion intervals (the percentage of total interinfusion intervals occuring during each 15 minute division) permitted the discrimination between CSF and low, high and optimum cocaine concentrations (Fig. 1) since significantly different patterns of responding were maintained by each (Table 1). For example, when a high dose was delivered, 55% of the interinfusion intervals were less than 15 minutes long while only 42% fell within this range at low doses. In addition, only 11% of the intervals were longer than 45 minutes with high doses compared to 33% at lower concentrations. CSF, high, low and optimum cocaine concentrations could be easily distinguished from one another by determining the relative frequency of interinfusion intervals less than 15 minutes (division 1) and longer than 45 minutes (division 4).

The 6-OHDA lesion at the site of self-administration reduced drug-intake to vehicle levels (36%) and flattened the dose-response curves of all four animals (Fig. 2). The patterns of responding with the optimum concentration of cocaine (determined pre-lesion) were consistent with those observed with CSF and low doses (Fig. 1 and Table 2). These effects were not transient since pre-lesion rates of self-administration did not return during the 6 to 8 weeks post-lesion that the behavior was monitored. Responding could not be reinitiated by either increasing or decreasing the cocaine concentration. The substitution of dopamine (300 pmol) for cocaine resulted in a rapid re-acquisition of lever-pressing (Fig. 2) almost to pre-lesion baseline levels (88%) with rates and patterns of responding similar to those maintained by the high or optimum dose of cocaine prior to the lesion (Fig. 1 and Table 2). This dopamine self-administration was probably mediated through postsynaptic D_2-dopaminergic receptors since blockade with the co-infusion of sulpiride again reduced intake to vehicle levels. Serotonergic neurons potentially damaged by the 6-OHDA lesion were probably not involved since the substitution of equimolar concentrations of this neurotransmitter would not maintain responding in the lesioned animals.

Histological assessment demonstrated that the guide cannulae were localized within the right medial prefrontal cortex

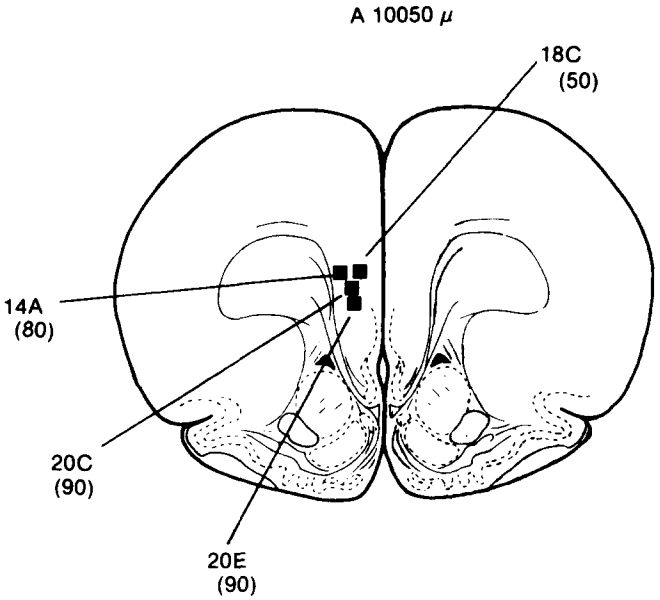

FIG. 3. Location of the injection cannulae tips in the medial prefrontal cortex and the optimum dose of cocaine for each rat.

(Fig. 3) with apparent neuronal degeneration contained within 1 mm of the cannulae tips. The 6-OHDA lesions resulted in significant decreases in the content of DA (−45%), DOPAC (−34%) and 5-HT (−46%) in the right medial prefrontal cortex (lesioned site) compared to the left, but did not affect the content of NE (Table 3). No significant changes were observed in the content of these compounds in the right and left nucleus accumbens, further demonstrating that the lesion was localized within the right side medial prefrontal cortex and also suggesting that this population of dopaminergic fibers does not send collaterals to the nucleus accumbens.

The apparent decrement in 5-HT in the right medial prefrontal cortex after the lesion was not an actual decrease. In a separate investigation (unpublished observations), five rats were trained to self-administer cocaine into the medial prefrontal cortex using the same procedures described in the Method section of this report. When stable baselines of

drug-intake were obtained, each animal was implanted with an indwelling jugular catheter under sodium pentobarbital/methylatropine sulfate anesthesia for pulse labeling with radioactive precursors for monoamine and amino acid neurotransmitters. The animals were allowed to intracranially self-administer cocaine for an additional six weeks and were then sacrificed by total immersion in liquid nitrogen. 5-HT content at the self-administration site in these non-lesioned animals with a similar history of medial prefrontal cortex cocaine self-administration was not different from that in the same region of the lesioned animals (30.8±6.4 pmoles/mg protein vs. 29.6±5.6 pmol/mg protein). However, the content on the left side was significantly increased (164%) in lesioned animals (53.0±5.7 pmol/mg protein) compared to these non-lesioned rats (32.4±7.5 pmol/mg protein), suggesting a decreased release or possible hyperinnervation contralateral to the lesion. The ratio of the content of DA to DOPAC in the right medial prefrontal cortex (1.09) (a crude estimate of the turnover rate of DA) was significantly lower (−62%) compared to the left side (2.85) of the non-lesioned rats. This asymmetry in DA turnover was not observed in the lesioned animals. The lesion also appears to have affected neurotransmission in the nucleus accumbens. The content of DOPAC was significantly reduced in the right (−32%) and left (−30%) nucleus accumbens of the lesioned rats (34.5±8.0 and 32.5±1.7 pmol/mg protein) compared to the non-lesioned animals (50.9±7.0 and 40.6±10.0 pmol/mg protein), while the content of DA was not significantly different. Therefore, the ratio of DA to DOPAC in the right (10.22) and left (11.97) nucleus accumbens was significantly increased (162% and 201%, respectively) in lesioned animals compared to non-lesioned rats (6.31 and 5.96, respectively). The content of 5-HT was also significantly increased in the right (177%) and left (188%) nucleus accumbens of lesioned rats (70.8±12.8 and 68.9±13.9 pmol/mg protein) compared to non-lesioned controls (40.0±8.0 and 36.6±10.4 pmol/mg protein). These data suggest that, while the lesion directly affected DA content in the medial prefrontal cortex, neurotransmission involving other neurotransmitters and another brain region was also significantly altered.

DISCUSSION

6-Hydroxydopamine lesions of the medial prefrontal cortex eliminated intracranial cocaine self-administration into this region. Dose-response analyses demonstrated that the drug no longer maintained responding above pre-lesion vehicle levels at any concentration tested. The substitution of dopamine (but not serotonin) for cocaine re-established pre-lesion rates and patterns of drug-intake, and this self-administration was attenuated by the co-infusion of a D_2-dopaminergic receptor antagonist. These data suggest a direct presynaptic action of cocaine on nerve endings that may be associated with dopaminergic reuptake sites. The resulting increased synaptic concentration of dopamine may be necessary for maintenance of cocaine self-administration into the medial prefrontal cortex. Even though only one dose of serotonin was tested, the patterns of responding with this dose (equivalent to the effective dose of dopamine) were similar to vehicle, not to the optimum or high dose of cocaine. Thus, serotonin did not appear to initiate any discernable level of reinforcing neuronal activity.

This attenuation of intracranial cocaine self-administration could result from several potential effects of the 6-OHDA lesions. The lesion could decrease the ability of the animals to complete the appropriate response. However, this is unlikely since operant levels of responding were not affected and dopamine microinfusions post-lesion maintained pre-lesion rates of self-administration. Furthermore, similar lesions of the medial prefrontal cortex in animals trained to respond under a fixed-interval 2-minute schedule of food reinforcement produced a slight nonsignificant increase rather than a decrease in control rates of responding [11]. The effects of the lesion probably did not result from an alteration in the effects of the drug on motor activity since intracranial cocaine self-administration does not result in a non-specific increase in activity [17]. The animals effectively maintained their pre-lesion body weights throughout the period of assessment following the lesion, indicating that alterations in food-intake and levels of deprivation were also not likely responsible for these effects. The effects of the lesion could have resulted from neuronal damage at sites distal to the self-administration locus or from inadequate damage at the lesion site. However, the volume and rate of neurotoxin infusion make the former conclusion unlikely. Histological and neurochemical evaluation showed the lesion to be localized within the medial prefrontal cortex. *In vivo* experiments assessing drug diffusion have also shown non-contingently infused [^{3}H]-cocaine to be localized with 1 mm of the injection site in the medial prefrontal cortex [21], also suggesting that the reinforcing effects of the drug are restricted to this region. In addition, if the lesion had only resulted in a partial loss of involved neurons, then a shift in the dose response curve rather than a direct flattening of the curve would be expected. These results suggest that a significant population of neurons necessary for the initiation of reinforcing neuronal activity had been detrimentally affected. The number of behavioral sessions required for the completion of this investigation is probably not a factor in the results since rats maintain stable rates of intracranial cocaine self-administration into the medial prefrontal cortex for more than 120 behavioral sessions [20]. The anesthetic used during the lesion is also not likely to have affected self-administration since animals trained under conditions similar to those outlined in this report and implanted with indwelling jugular catheters under the same anesthesia rapidly reacquired lever pressing following surgery (unpublished observations).

The delineation of the neuronal systems that initiate and/or mediate drug reinforcement is important. The receptors through which cocaine directly activates reinforcing neuronal activity may represent initiation sites, while mediation circuits that participate in general reinforcement processes (as demonstrated by intracranial electrical self-stimulation) may be subsequently recruited for the full expression of this activity. Different reinforcers could result in the activation of similar components of these mediation circuits. The distinction between reinforcing stimuli could be partially dependent upon which discrete inputs to these circuits are activated. The magnitude of reinforcement that follows could be related to the frequency, duration and spread of this neuronal activity within these mediation circuits which could be modulated by motivational variables (e.g., deprivation, satiation, etc.) or behavioral history.

6-Hydroxydopamine lesions of the medial prefrontal cortex appear to reduce or eliminate the reinforcing efficacy of cocaine self-administration at this site. Similar lesions of the nucleus accumbens disrupt intravenous self-administration [37], but the behavior recovers to pre-lesion levels unless a sufficient dopamine loss (−80%) is achieved [38]. Lesions of

the ventral tegmental area also affect self-administration, but the degree of attenuation does not correlate with the reduction of dopamine content in the nucleus accumbens [39], suggesting that dopaminergic innervations of other structures are necessary. Previous research has demonstrated that the response-dependent delivery of cocaine into the medial prefrontal cortex will initiate reinforcing neuronal activity [17]. The involvement of postsynaptic components in these processes was suggested since self-administration was blocked with a dopaminergic D_2 receptor antagonist but not with dopaminergic D_1, muscarinic-cholinergic or beta-noradrenergic receptor antagonists [20]. D_2 dopaminergic receptors appear to be excitatory to these processes since patterns of drug-intake after blockade were similar to those observed with CSF or low cocaine doses [20]. The results presented here demonstrate that dopaminergic innervations are necessary for the initiation of reinforcing neuronal activity by cocaine in the medial prefrontal cortex. The drug probably interacts with presynaptic dopaminergic reuptake sites to inhibit neuronal reuptake [28]. The increased synaptic concentration of dopamine could then result in a potentiation of activity of postsynaptic D_2 receptors to initiate reinforcing neuronal activity. The 6-hydroxydopamine lesions damaged presynaptic dopaminergic terminals and eliminated the primary mechanism for cocaine-induced initiation of reinforcing neuronal activity. Post-lesion response-contingent microinfusions of dopamine appear to activate postsynaptic neuronal systems in a manner analogous to the delivery of cocaine pre-lesion. Dopaminergic nerve endings therefore appear necessary for the initiation of reinforcing neuronal activity by cocaine in the medial prefrontal cortex. However, this does not exclude alternative mechanisms in other brain regions that may also be directly affected by systemic administration of the drug.

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