Pharmacology Biochemistry & Behavior, Vol. 22, pp. 427–434, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Monoaminergic and Local Anesthetic Components of Cocaine's Effects on Kindled Seizure Expression

R. D. RUSSELL[1] AND J. S. STRIPLING[2]

Department of Psychology, University of Arkansas, Fayetteville, AR 72701

Received 18 April 1984

RUSSELL, R. D. AND J. S. STRIPLING. *Monoaminergic and local anesthetic components of cocaine's effects on kindled seizure expression.* PHARMACOL BIOCHEM BEHAV **22**(3) 427–434, 1985.—Male Long-Evans rats were kindled via daily electrical stimulation of the left prepyriform cortex. The animals were then used in two experiments which examined the pharmacological basis of cocaine's effects on three mutually exclusive components of the kindled seizure, which were the following: (a) latency to clonus, (b) clonus duration, and (c) duration of AD outlasting clonus. The first experiment compared the effects produced by cocaine HCl (20 mg/kg, IP), lidocaine HCl (20 mg/kg, IP), and amphetamine sulfate (2.5 mg/kg, IP). The results indicated that both cocaine and lidocaine reduced the duration of kindled AD, latency to clonus, and duration of AD persisting beyond clonus, thus suggesting that these cocaine effects are mediated by local anesthetic mechanisms. Only cocaine reduced clonus duration, which suggests that this cocaine effect is not produced by a local anesthetic action. The second experiment examined the effects of cocaine following the administration of three dose levels of the monoamine antagonists haloperidol, prazosin, yohimbine, propranolol, or metergoline (selected for their ability to block dopamine, alpha-1-norepinephrine, alpha-2-norepinephrine, beta-norepinephrine, and serotonin receptors, respectively). The results of this experiment found no support for a monoaminergic contribution to cocaine's effect on clonus latency or AD after clonus. However, results for prazosin, which reduced clonus duration and exhibited an additive effect with cocaine on this variable, suggest that cocaine's norepinephrine action (especially on the alpha-norepinephrine systems) may modulate clonus duration.

Kindling Prepyriform cortex Rat Cocaine Lidocaine Amphetamine Haloperidol
Prazosin Yohimbine Propranolol Metergoline

SUFFICIENTLY intense electrical stimulation of a limbic brain site can readily trigger a seizure afterdischarge (AD) which does not propagate far from the site of stimulation. "Kindling" refers to a gradual intensification of the AD (increasing amplitude, duration, and propagation) and the eventual emergence of a behavioral clonic convulsion which accompanies the AD as a result of repeated elicitation of ADs [11,24]. The clonic convulsion is produced when the AD propagates far enough to activate those areas of the brain which drive the behavioral convulsion. For some limbic sites, and perhaps all, kindling develops further so that multiple episodes of clonus with rearing and falling, wild running, and mild tonic limb extension accompany the AD [23]. Eventually, after hundreds of ADs elicited over the course of months, behavioral convulsions occur spontaneously [23]. This fact suggests that the kindled seizure may not reach full maturity, if in fact it ever does, until convulsions occur spontaneously. However, AD and behavioral convulsion durations are relatively stable for several hundred stimulations prior to the time that spontaneous convulsions emerge [23]. Consequently, the term "limbic kindled" will be used to refer to a limbic AD that has developed so that a clonic convulsion is triggered reliably, even though it may not be fully developed.

The limbic kindled seizure has been used for screening potential anticonvulsant drugs (e.g., [1, 3, 4]). The precise control which the kindled-seizure model provides over time of occurrence and anatomical origin of the seizure makes the model attractive for the study of acute drug effects on seizure mechanisms. In addition, the kindled seizure allows simultaneous study of separate seizure parameters such as seizure propagation and persistence of seizure activity.

Paradoxically, cocaine produces both proconvulsant and anticonvulsant effects on the limbic kindled AD. The anticonvulsant effects include a reduction of the AD duration kindled from a variety of rat [34] and cat [16, 18, 19] limbic sites. Cocaine reduces the AD duration recorded at the site of stimulation or in secondary sites [18]. Cocaine also increases the kindled AD threshold for high-frequency electrical stimulation of limbic or cortical sites [19, 35, 36]. Cocaine's facilitative effects on kindled seizure expression include a decrease in AD threshold for low stimulation fre-

[1]R. D. Russell submitted this research to the Department of Psychology of the University of Arkansas in partial fulfillment of the requirements for the Master of Arts degree.

[2]Requests for reprints should be addressed to J. S. Stripling.

quencies [16, 18, 35], an acceleration of the propagation of seizure activity from the site of stimulation to distant sites [17], and a reduction of clonus latency, which has been described as the behavioral manifestation of cocaine's facilitation of seizure propagation [34].

It has been difficult to explain the various antagonistic and facilitative effects that cocaine produces on the kindled seizure using a single mechanism. Cocaine is known to have two prominent pharmacological actions, those being monoamine reuptake blockade [28,29] and local anesthesia [9,27]. Cocaine's paradoxical reduction of AD duration and clonus latency have been suggested to be manifestations of cocaine's monoaminergic and local anesthetic actions, respectively [34]. The following two studies examine the pharmacological basis of cocaine's effects on the seizure kindled from the left prepyriform cortex (PPC). The studies also examine the possibility that cocaine's paradoxical effects result from its separate pharmacological actions. Experiment 1 compares the effects produced by cocaine with those produced by lidocaine and amphetamine. Experiment 2 examines various monoaminergic receptor antagonists for their ability to produce effects on the kindled PPC seizure or to block cocaine's effects.

EXPERIMENT 1

METHOD

Subjects

The subjects were male Long-Evans rats (Blue Spruce Farms) which weighed 315–390 g at the time of surgery. Rats were housed individually in clear plastic cages containing nonaromatic bedding (Aspen Bed). The rats were given free access to Purina Rat Chow and tap water. The rat colony room was maintained on a 12 hr/12 hr light-dark cycle and all data were collected during the light period.

Surgery

The rats were anesthetized with sodium pentobarbital (42.5 mg/kg, IP) and chloral hydrate (100 mg/kg, IP), with atropine sulfate (4 mg/kg, IP) administered to inhibit congestion. The basic surgical procedure has been described [33]. Briefly, a monopolar electrode (200 μm enamel-insulated stainless steel) was implanted into the left PPC (2.4 mm anterior and 2.8 mm lateral to bregma, 7.8 mm ventral to dura with a 14° lateral angle). Stainless steel screws inserted over the anterior and posterior right cortex served as stimulation and recording references, respectively. Postoperative recovery of 11 to 23 days was allowed before kindling began.

Apparatus

The rat and its home cage were placed into a clear acrylic chamber which was contained within a Faraday cage and a larger plywood chamber. The stimulation/recording cable was coupled to a slip-ring commutator (BRS/LVE) and the assembly was mounted on a counterbalanced arm, giving the rat full freedom of movement. Convulsive behavior was monitored with closed-circuit television and recorded on video tape. Electrographic seizures were recorded with a Beckman R611 polygraph. Constant current stimulation was provided by a Grass PSIU6 stimulus isolation unit and a Grass S48 square wave stimulator. A logic-controlled relay disconnected the rat's leads from the polygraph during the stimulation train.

Procedure

For kindling and post-kindling drug tests, the PPC was stimulated with a 2 sec train of cathodal square-wave current pulses (0.2 msec pulse width) delivered at 50 pps. Pre-kindling AD thresholds were determined via an ascending stairstep procedure (beginning at 10 μA with 10 μA steps every 60 sec until an AD was elicited) on the first day. Animals which had an AD threshold greater than 200 μA were not kindled. During kindling, the rats were used to study the effect of stimulation intensity on the development of kindling (Russell and Stripling, manuscript in preparation). Stimulation intensity during kindling varied from the pre-kindling AD threshold value to 200% of the threshold. Animals were kindled by daily stimulation to a criterion of five consecutive ADs with forelimb clonus. Post-kindling AD thresholds were then determined using a procedure identical to that used for assessing pre-kindling thresholds. Three days later an AD was elicited in each animal via stimulation at a current intensity of 200% of the post-kindling AD threshold or 100 μA minimum. Drug testing began three days later.

Drug tests were conducted at three day intervals with no stimulation delivered between successive tests. The drugs tested were physiological saline, 2.5 mg/kg d-amphetamine sulfate (Sigma), 20 mg/kg cocaine HCl (Merck), and 20 mg/kg lidocaine HCl (Astra). All injections were intraperitoneal. Each animal was tested once in each drug condition. The order of drug administration was counterbalanced across animals.

For each drug test, the animal was given the appropriate injection and then an AD was elicited either 15 min (for saline, cocaine, and lidocaine tests) or 30 min (for amphetamine tests) following the drug injection. ADs were triggered by stimulating the animal with a current intensity twice that of the individual's post-kindling AD threshold or 100 μA minimum (Mean stimulation current$\pm$S.E.M.$=$ 122$\pm$5 μA). On 12 occasions (7 cocaine and 5 lidocaine tests) the stimulation did not elicit an AD greater than 10 sec accompanied by forelimb clonus, and the current was increased 10 μA and redelivered at 60 sec intervals until an AD greater than 10 sec was elicited.

Data Analysis

Forty-three animals met the kindling criterion and began Experiment 1. Data were discarded for four animals: one animal lost its electrode assembly, one animal died of a lidocaine induced convulsion, one animal died of a cocaine induced convulsion, and one animal did not exhibit an AD greater than 10 sec for one of the drug tests. All of the remaining 39 animals had a long AD with strong forelimb clonus (the minimum forelimb clonus duration observed was 18 sec) for each drug test. The data were analyzed with one-way ANOVAs for repeated measures followed by specific comparisons using the Newman-Keuls' test.

Drug effects on the AD and behavioral convulsion were analyzed separately by dividing the AD, recorded from the PPC, into three separate and mutually exclusive components: (a) the latency to the behavioral convulsion (operationally defined as forelimb clonus), which represents the speed of seizure propagation from the PPC to the areas of the brain involved in generating the behavioral convulsion; (b) the duration of the behavioral convulsion, which represents the duration of seizure activity propagated to and sustained in those areas of the brain generating the behavioral convul-

TABLE 1

EXPERIMENT 1: EFFECTS OF DRUG TREATMENT ON THE DURATION (SEC) OF THE PREPYRIFORM CORTEX KINDLED AD AND ITS VARIOUS COMPONENTS

Drug	AD Duration	Clonus Latency	Clonus Duration	AD Duration After Clonus
Saline	86.0 ± 5.4	5.7 ± 0.5	58.1 ± 3.0	22.2 ± 4.1
Amphetamine (2.5 mg/kg)	81.3 ± 5.8	6.7 ± 0.6*	52.5 ± 2.7	22.0 ± 3.9
Lidocaine (20 mg/kg)	69.2 ± 3.8*	3.4 ± 0.5*‡	59.7 ± 1.9	6.2 ± 2.6*‡
Cocaine (20 mg/kg)	52.1 ± 2.6*†‡	1.0 ± 0.4*†‡	47.2 ± 2.0*†	4.0 ± 1.1*‡

Note: Values are means ± S.E.M. (N=39). Specific comparisons between drug groups used the Newman-Keuls' test ($p<0.05$).
*Significantly different from saline.
†Significantly different from lidocaine.
‡Significantly different from amphetamine.

sion; and (c) the duration of AD outlasting the primary episode of forelimb clonus, a proposed measure of local seizure persistence.

RESULTS

Movement arrest, chewing, or head nodding were observed during the early period of the AD (i.e., before forelimb clonus). The most prominent behavioral feature of the AD was bilateral and synchronous forelimb clonus, which was observed during every AD. Some, but not all, animals exhibited one or more rearing and falling episodes occurring during forelimb clonus. Unilateral clonus of the ipsilateral or contralateral forelimb usually occurred near the end of forelimb clonus. The AD after clonus was expressed predominantly by stillness or spasmodic walking. Wild running or tonic limb extension were never observed during the AD.

Drug effects are presented in Table 1. There was a significant drug effect on AD duration, $F(3,114)=15.44$, $p<0.01$. Both cocaine and lidocaine significantly reduced the duration of the AD, with cocaine being significantly more effective than lidocaine. A similar pattern of results was observed for forelimb clonus latency; a significant drug effect was found, $F(3,114)=57.21$, $p<0.01$, with cocaine and lidocaine significantly reducing the latency to forelimb clonus (cocaine was significantly more effective than lidocaine). In addition, amphetamine produced a small but significant increase in latency to forelimb clonus. The duration of forelimb clonus was affected by drug treatment, $F(3,114)=6.73$, $p<0.01$, but only cocaine significantly reduced this component. A significant effect on the duration of AD after forelimb clonus was observed, $F(3,114)=13.37$, $p<0.01$. Both cocaine and lidocaine significantly reduced this component.

EXPERIMENT 2

METHOD

Procedure

Testing of animals in Experiment 2 began three days following completion of Experiment 1. The subjects were the 40 rats that completed Experiment 1. The electrical stimulation settings were the same as those used in Experiment 1.

The neurotransmitter antagonists tested were haloperidol (McNeil), propranolol HCl (Sigma), yohimbine HCl (Sigma), prazosin HCl (Pfizer), and metergoline (Farmitalia Carlo Erba): these drugs were selected for their ability to block dopaminergic, beta-noradrenergic, alpha-2-noradrenergic, alpha-1-noradrenergic, and serotonergic receptors, respectively. Each of the antagonists was prepared in four dose levels from salts except haloperidol, which was diluted with physiological saline to obtain lower doses from a commercially available solution (Haldol; 5 mg/ml). The control was an injection of the appropriate vehicle. The vehicle for haloperidol, yohimbine, and propranolol was physiological saline. The metergoline vehicle was a solution of 0.7% ascorbic acid in sterile water. A suspension vehicle composed of 2% gum acacia in sterile water was used for prazosin. The doses for the antagonists were the following: 0.04, 0.2, and 1.0 mg/kg for yohimbine and metergoline; 0.1, 0.5, and 2.5 mg/kg for haloperidol; and 0.2, 1.0, and 5.0 mg/kg for propranolol and prazosin.

The administration procedure for each drug test was an injection (IP) of the appropriate dose of the appropriate antagonist followed by an injection of either 15 mg/kg cocaine HCl or physiological saline. All antagonist injections preceded the cocaine (COC) or saline (SAL) injection by 15 minutes except for metergoline, which preceded the second injection by 45 minutes. Electrical stimulation at the same current intensity used in Experiment 1 was administered 15 minutes following the COC or SAL injection. If no AD was elicited the current was increased 50 μA (followed by an increase of 100 and 200 μA if necessary) and redelivered 60 seconds later.

The 40 rats were assigned, via block randomization as they completed Experiment 1, for testing with one of the 5 antagonist drugs. This resulted in eight animals per antagonist group. After the rats completed testing on their first antagonist (block 1), they were tested with a second antagonist (block 2). The eight subjects for a specific antagonist in block 2 were two subjects from each of the other four antagonist drugs tested in block 1. Testing each of the animals on two antagonists resulted in 16 animals per antagonist group.

Drug tests were conducted at three day intervals. Each dose of an antagonist or the vehicle injection was tested with both COC and SAL before testing of the next dose. COC and SAL test days were alternated, thus giving six days separating successive COC injections. Each subject received a total of 16 tests (3 antagonist doses and 1 vehicle control × 2

TABLE 2

EXPERIMENT 2: EFFECT OF PRAZOSIN AND COCAINE ON THE DURATION (SEC) OF THE PREPYRIFORM CORTEX KINDLED AD AND ITS COMPONENTS

Dependent Variable	Prazosin dose (mg/kg) vehicle	0.2	1.0	5.0
AD duration				
Saline	90.8 ± 7.1	93.3 ± 5.8	94.4 ± 7.5	85.0 ± 7.6
Cocaine (20 mg/kg)	93.5 ± 13.2	77.5 ± 7.3	62.9 ± 7.2*‡	49.3 ± 6.6*‡
Forelimb clonus latency				
Saline	5.5 ± 0.8	5.9 ± 1.3	5.7 ± 0.9	7.0 ± 1.3
Cocaine (20 mg/kg)	0.8 ± 0.6	1.9 ± 0.8	0.4 ± 0.6	0.5 ± 0.5
Forelimb clonus duration				
Saline	51.3 ± 4.0	49.1 ± 3.0	43.4 ± 3.1	39.4 ± 3.4†
Cocaine (20 mg/kg)	50.7 ± 4.1	35.2 ± 1.4*‡	36.2 ± 1.7‡	31.0 ± 1.7‡
AD following clonus				
Saline	34.1 ± 6.6	38.3 ± 6.4	45.3 ± 7.4	37.3 ± 7.0
Cocaine (20 mg/kg)	42.1 ± 10.9	40.5 ± 6.7	26.3 ± 6.7	17.8 ± 5.6‡

Note: The values represent means ± S.E.M. (N=15). Main effects were produced by cocaine on forelimb clonus latency and by prazosin on forelimb clonus duration. Statistically significant prazosin × cocaine interactions were found for all analyses except forelimb clonus latency. The statistical tests ($p<0.05$) indicated below in parentheses were used for specific comparisons between means when the ANOVA indicated an interaction.

*Significantly different from saline with same dose of prazosin (Tukey's).

†Significantly different from vehicle + saline (Newman-Keuls').

‡Significantly different from vehicle + cocaine (Newman-Keuls').

COC-SAL conditions × 2 antagonists). In order to dissociate time- or experience-related changes from possible antagonist dose effects, the dose order was counterbalanced across animals.

Three days after completion of testing in block 2, animals were given a saline injection and an AD was triggered to check the stability of the kindled seizure. Electrode placement was verified histologically using the Prussian Blue technique. Histology revealed that all electrode placements were in or near the area of the left PPC. No animals were excluded from the experiment due to electrode placement.

Data Analysis

The preplanned statistics were two-way ANOVAs (2×4; COC-SAL × antagonist dose) with repeated measures on both factors. Main effects for antagonist dose in the absence of an interaction with COC were analyzed with specific comparisons using the Newman-Keuls' test conducted on the data collapsed across both SAL and COC conditions. Significant interactions were analyzed with Tukey's test for unconfounded means to compare values with SAL to those with COC for each of the three doses of the antagonist and the vehicle injection, thus producing four specific comparisons per interaction. In cases with significant interactions, the effect of the antagonist alone was analyzed by using a one way ANOVA for repeated measures on the SAL data only and specific comparisons using the Newman-Keuls' test.

A total of 20 analyses were performed (5 antagonist groups × 4 dependent variables). In three analyses, data were subjected to a logarithmic transform because of pronounced nonhomogeneity of variance. One block of data was excluded from analysis for 6 animals because an AD greater than 10 sec accompanied by forelimb clonus was not observed for each drug test. One block of data for two animals was not analyzed because a convulsion was elicited by a cocaine injection. One animal died before completion of testing. These losses resulted in analyses conducted on data for 16, 15, 14, 13, and 14 subjects for the prazosin, yohimbine, propranolol, haloperidol, and metergoline groups, respectively.

RESULTS

None of the receptor antagonists blocked cocaine's effect on any of the dependent variables. In addition, none of the receptor antagonists significantly affected clonus latency and only two antagonists, prazosin and haloperidol, were effective on any of the other components. Overall, cocaine was reliably effective in reducing the forelimb clonus latency and forelimb clonus duration (i.e., these variables were significantly reduced by cocaine for every antagonist group).

Prazosin clearly interacted with COC on three of the dependent variables (see Table 2). COC and prazosin interacted in reducing the AD duration, $F(3,42)=4.65$, $p<0.01$, the forelimb clonus duration, $F(3,42)=3.82$, $p<0.05$, and the duration of AD after forelimb clonus, $F(3,42)=3.00$, $p<0.05$. COC significantly reduced the AD duration only when animals were given 1.0 or 5.0 mg/kg prazosin. COC significantly reduced clonus duration only when animals were given 0.2 mg/kg prazosin. COC did not significantly affect AD after clonus at any dose of prazosin; however, prazosin produced a dose-dependent reduction of AD after clonus but only with COC, $F(3,42)=4.25$, $p<0.05$. Prazosin administered in conjunction with SAL significantly affected only

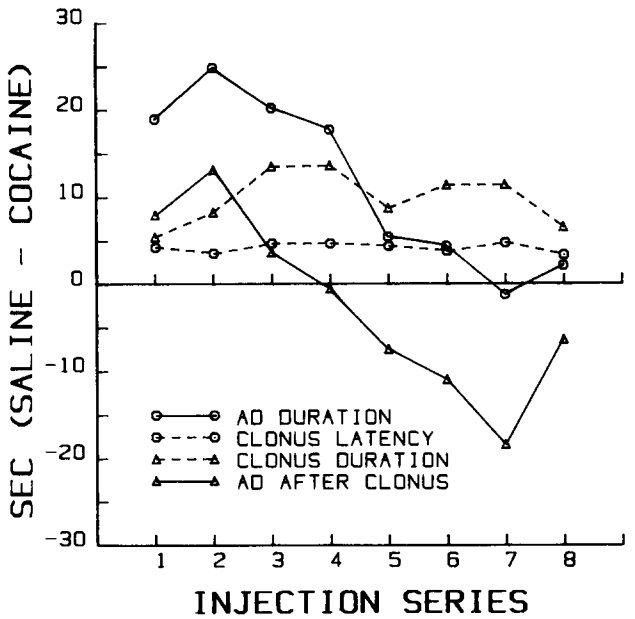

FIG. 1. Experiment 2. The effect of cocaine on various components of the kindled prepyriform cortex seizure across the series of 8 cocaine-saline comparison tests (16 injections). Difference scores are the mean duration (sec) for the antagonist + SAL condition minus the mean duration for the corresponding antagonist + COC condition. The data are for 32 animals which had ADs > 10 sec with forelimb clonus for every drug test.

clonus duration, $F(3,42)=4.38$, $p<0.01$, with the high dose producing a reduction.

Haloperidol was the only other antagonist which had a significant effect on the kindled seizure. Haloperidol reduced the AD duration, $F(3,36)=5.11$, $p<0.01$, and the AD after clonus, $F(3,36)=3.61$, $p<0.05$. Newman Keuls' tests revealed that only the highest dose of haloperidol produced significant reductions of these variables.

In contrast to the results of Experiment 1, COC's reduction of the AD duration and AD after clonus was unreliable (i.e., COC reduced AD duration for only 3 of the 5 antagonist groups and reduced AD after clonus for only one antagonist group). Data for all antagonist groups were combined and analyzed for 32 animals which had ADs greater than 10 sec for every drug test. The counterbalancing for antagonist dose allowed an interpretable analysis of the data using a two-way ANOVA for repeated measures (SAL + antagonist vs. COC + antagonist with eight repeated measures, four in block 1 and four in block 2). The analyses revealed that COC interacted with the series of eight SAL-COC comparisons for AD duration, $F(7,217)=2.81$, $p<0.01$, and AD after clonus, $F(7,217)=2.97$, $p<0.01$. Neither AD duration nor AD after clonus significantly changed over the course of the experiment for animals when tested with SAL. However, both AD duration and AD after clonus increased for the COC tests across the injection series, $F(7,217)=3.98$ and 6.38, $ps<0.01$ for AD duration and AD after clonus, respectively. In contrast, COC's effect on clonus latency and clonus duration remained stable from the beginning to the end of Experiment 2. Figure 1 illustrates the weakened COC effect on AD duration and AD after clonus and the stability of COC's effect on the other two variables.

The progressive weakening of COC's reduction of AD duration and reversal of its effects on AD after clonus reduces the likelihood of demonstrating an antagonism of COC's effect by a receptor antagonist, especially for the data collected in block 2. For this reason, the data were also analyzed using only the data collected in block 1 (first antagonist tested). These analyses, which had a maximum of eight subjects per anagonist group, also failed to demonstrate any consistent antagonism of any of COC's effects.

The progressive diminution of COC's reduction of the AD duration can be characterized as tolerance. However, tolerance development cannot explain the apparent reversal of COC's effect on the AD after clonus which accompanied the loss of COC's effect on AD duration. A possible explanation is that the seizure changed (i.e., kindled further) over the course of the experiments so that the seizure became resistant to COC's suppressive effect during the later stages. Four data points for the various control tests over the course spanned by the experiments (Experiment 1 SAL, Experiment 2/block 1 vehicle + SAL, Experiment 2/block 2 vehicle + SAL, and post-Experiment 2 stability test) were analyzed to determine if a change had occurred in the non-drugged seizure. ANOVAs did not reveal any significant change in the AD or any of its components (see Table 3). But, because many animals (especially in Experiment 1) did not demonstrate a persistent local seizure (i.e., an AD outlasting the primary episode of clonus), the data for AD after clonus were dichotomized (scored as either present or absent) and analyzed with the nonparametric Cochran's Q test [30]. The test indicated that the probability of elicitation of a persistent local seizure increased over the course of the experiments, $Q(3)=20.25$, $p<0.001$. In addition, the behavioral expression of the AD after clonus appears to have changed. During Experiment 2, but not in Experiment 1, some animals began exhibiting bizarre behaviors (e.g., rapid running, sudden and wild jumping, and climbing or biting the cage wall) during the persistent local seizure component of the AD and the immediate (i.e., less than 60 sec) post-ictal period. Also, during the post-ictal period many animals began exhibiting brief bursts (1–5 sec) of clonus, which were sometimes but not always triggered by approaching the animal to remove it from the chamber. However, tonic extension was never observed during the AD or post-ictal period.

DISCUSSION

Cocaine's reduction of the duration of the limbic kindled AD is well documented [16, 18, 19, 34], but it has not been established which of cocaine's neuropharmacological actions (local anesthesia or monoamine reuptake blockade) is responsible for this effect. Data relating to monoaminergic mediation of this effect are not consistent. Monoaminergic mediation of cocaine's reduction of limbic AD duration is supported by other research showing alterations in AD duration produced by other drugs which modify monoaminergic transmission [10, 12, 14, 20, 31]. However, other data are inconsistent with monoaminergic mediation of AD duration. For example, amphetamine has had no effect on limbic kindled (Experiment 1) or cortical kindled AD [6]. Also, none of the receptor antagonists in Experiment 2 blocked cocaine's effect on AD duration. Massive depletion of norepinephrine, which facilitates the development of kindling [22], has no effect on limbic AD duration after kindling has developed [37]. In addition, data relating to local anesthetic mediation of cocaine's reduction of limbic kindled AD are not entirely

TABLE 3

DATA FOR CONTROL CONDITIONS ACROSS EXPERIMENTS 1 AND 2 (N=32)

Dependent Variable	Time of testing			
	Expt. 1	Expt. 2 Antagonist I	Expt. 2 Antagonist 2	Post-Expt. 2
AD duration (sec)	85.4 ± 6.2	95.8 ± 4.0	97.0 ± 4.5	93.5 ± 3.4
Clonus latency (sec)	5.4 ± 0.6	5.4 ± 0.6	5.8 ± 0.7	6.6 ± 1.1
Clonus duration (sec)	56.4 ± 3.4	59.7 ± 2.9	58.9 ± 2.3	55.4 ± 1.8
AD after clonus (sec)	23.6 ± 4.6	30.1 ± 4.6	32.4 ± 4.4	31.6 ± 3.3
Percentage of ADs outlasting clonus	63.0%	85.0%	97.0%	97.0%

Note: None of the duration measures changed significantly over the course of the experiments. However, the probability of elicitation of a persistent local seizure (AD after clonus greater than zero) increased significantly over the time spanned by the data points.

consistent. Previous studies, with smaller sample sizes than Experiment 1, have not reported limbic AD reduction with lidocaine [34] or procaine [25] although several have reported that these local anesthetics reduce the duration of cortical kindled AD [6,26]. Lidocaine reduced the duration of AD in Experiment 1. Taken as a whole, these data do not provide a clear indication of the pharmacological basis of cocaine's reduction of the duration of limbic kindled AD.

To achieve a better understanding of cocaine's effect on the limbic kindled seizure and the pharmacological basis of its effects, we have divided the AD into three mutually exclusive components which represent the following conceptually distinct events: (a) the latency to forelimb clonus, which is a measure of the speed of seizure propagation from the area of stimulation (PPC) to those neural systems which drive clonus; (b) the duration of the primary episode of forelimb clonus, which is a measure of seizure duration in those systems which drive clonus; and (c) the duration of AD outlasting the primary episode of forelimb clonus, which is a measure of the persistence of the local (i.e., at the site of stimulation) electrographic seizure. Analyzing separately cocaine's effects on the different components of the AD suggests that cocaine's effect on the AD is due to several distinct effects based partly on separate pharmacological actions. Cocaine's local anesthetic action appears to be strongly implicated in its reduction of clonus latency and AD after clonus, whereas cocaine's monoaminergic action may be involved in its reduction of clonus duration.

A prominent effect produced by cocaine is its acceleration of the propagation of seizure activity from the site of origin to distant sites [17]. This effect is expressed behaviorally in decreased clonus latency [34], which measures the time for the seizure to propagate to the systems which produce clonus. Cocaine's reduction of clonus latency appears to be highly reliable (e.g., it was found for all antagonist groups in Experiment 2). Lidocaine produced a similar reduction of clonus latency ([34]; Experiment 1) whereas amphetamine produced a small increase in clonus latency (Experiment 1). These data, supported by the lack of effect of the monoamine antagonists on either clonus latency or cocaine's reduction of clonus latency (Experiment 2), support strongly a local anesthetic mediation of this variable.

In Experiment 1, both cocaine and lidocaine, but not amphetamine, produced a large reduction of local seizure persistence, suggesting that this effect is of local anesthetic origin. Although the high dose of haloperidol also reduced local seizure persistence (Experiment 2), it did not interact with cocaine and therefore is not supportive of monoaminergic mediation of cocaine's effect on this component.

Cocaine's reduction of clonus duration does not appear to be mediated by local anesthetic mechanisms. The observation that lidocaine had no effect on clonus duration, but had strong effects comparable to cocaine on the other two components of the AD, suggests that cocaine's reduction of clonus is pharmacologically different from its effect on the other two components. Although it is possible that a different dose of lidocaine might have produced a reduction of clonus, several observations suggest that cocaine and lidocaine produce equipotent central local anesthetic effects. The two drugs are equipotent in producing olfactory spindles [32], increasing cortical kindled AD threshold, reducing the duration of the focal component of cortical kindled AD ([36], Stripling and Russell, submitted for publication), and reducing AD after clonus in Experiment 1. In addition, the doses of cocaine and lidocaine used in Experiment 1 were both near the convulsive threshold (1 convulsion was produced by each drug), and consequently significantly higher doses of lidocaine could not have been used. Thus, the available evidence suggests that lidocaine acts selectively to reduce clonus latency and AD after clonus without a significant effect on clonus duration.

Cocaine's reduction of clonus duration may result from its monoaminergic, and more specifically, its alpha-noradrenergic actions. For example, several laboratories have reported that drugs which affect noradrenergic transmission produce effects on clonus duration [2, 4, 8, 20, 31]. In addition, amphetamine produced a trend toward shortening the duration of clonus in Experiment 1. In Experiment 2 prazosin, classified as an alpha-1 receptor antagonist [15], reduced clonus duration when administered alone but had no effect on clonus latency or AD after clonus. This selective effect of prazosin on clonus duration suggests that the alpha-noradrenergic system may modulate clonus duration. This interpretation is consistent with the dose dependent reduction of AD duration [12] and clonus duration [2] with the alpha-2 receptor agonists xylazine and clonidine, respectively. The results from Experiments 1 and 2 and those reported by others suggest that noradrenergic transmission af-

fects the duration of clonus. However, it should be pointed out that other research finds no support for noradrenergic involvement in limbic kindled AD or behavioral convulsion duration [37].

The only antagonist which influenced the effect of cocaine on clonus duration or any other component of the AD was prazosin. Prazosin's reduction of clonus duration summed with cocaine's effect. However, prazosin's interaction with cocaine was not selective to the clonus duration component (i.e., it interacted on AD duration and AD after clonus). These interactions suggest that monoaminergic transmission may modulate cocaine's effects. However, a pharmacokinetic source for this modulation cannot be ruled out. For example, prazosin, as a result of blocking postsynaptic alpha-adrenoceptors, produces vasodilatation and antagonism of norepinephrine produced vasoconstriction [5]. Cocaine, as a result of its effects on norepinephrine transmission, produces vasoconstriction which slows its own absorption [27]. Thus prazosin may have increased cocaine's absorption from the site of injection, and hence its availability to the brain. This model accounts for prazosin's potentiation of cocaine's effects mediated either by monoaminergic or local anesthetic actions, whereas the interactions can not be explained simply by prazosin blocking cocaine's monoaminergic actions.

Paradoxically, cocaine produces seizure antagonistic (e.g., decreased AD duration) and seizure facilitative (e.g., facilitation of seizure propagation) effects at a single dose and within a single seizure episode. Stripling and Hendricks [34] suggested that the conflicting nature of these effects might be attributable to separate pharmacological mechanisms. Although this explanation can account for cocaine's simultaneous facilitation of seizure propagation (local anesthetic) and reduction of clonus duration (monoaminergic), it can not explain completely the paradoxical nature of cocaine's effects. For example, cocaine's facilitation of seizure propagation (a seizure facilitative effect) and its reduction of seizure persistence (a seizure antagonistic effect) appear to be mediated by the same pharmacological action (local anesthesia). In discussing the paradoxical nature of cocaine's effects, Lesse and Collins [17] suggested that cocaine's reduction of AD duration may be causally related to excitatory effects of cocaine which facilitate the propagation of seizure activity. However, if these paradoxical effects are causally related then the effects must be caused by the same pharmacological action. The results from the present experiments provide evidence that cocaine's (and lidocaine's) reduction of AD duration is produced by the same pharmacological action as its facilitation of seizure propagation and reduction of local seizure persistence. Thus, a proposed causal relationship between cocaine's facilitation of seizure termination and seizure propagation [17] is strengthened.

Kindling development is associated with neurochemical deficits for various neurotransmitters [13]. It has been theorized that kindling or enhanced seizure excitability results from a reduction in the efficacy of a seizure-inhibiting neurotransmitter system (e.g., [7,21]). If a seizure can propagate to activate some seizure-inhibiting neurotransmitter system, then cocaine's facilitation of seizure propagation may cause its facilitation of seizure termination. In addition, if the deterioration of a seizure-inhibiting neurotransmitter system is produced by repetitive seizure propagation, then this process could be augmented by triggering the seizures in the presence of a drug which facilitates seizure propagation (i.e., cocaine). This could explain the progressive reversal of cocaine's effect on local seizure persistence as depicted in Fig. 1.

ACKNOWLEDGEMENTS

The authors thank M. Cunningham and C. Gramlich for their technical assistance, and Pfizer Laboratories and Farmitalia Carlo Erba for their generous gifts of prazosin and metergoline, respectively. This research was supported by a grant from the Marie Wilson Howells Fund and by BRSG S07 RR07101-04 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

REFERENCES

1. Albertson, T. E., S. L. Peterson and L. G. Stark. Anticonvulsant drugs and their antagonism of kindled amygdaloid seizures in rats. *Neuropharmacology* **19**: 643–652, 1980.
2. Ashton, D., J. E. Leysen and A. Wauquier. Neurotransmitters and receptor binding in amygdaloid kindled rats: Serotonergic and noradrenergic modulatory effects. *Life Sci* **27**: 1547–1556, 1980.
3. Ashton, D. and A. Wauquier. Behavioral analysis of the effects of 15 anticonvulsants in the amygdaloid kindled rat. *Psychopharmacology (Berlin)* **65**: 7–13, 1979.
4. Babington, R. G. and P. W. Wedeking. The pharmacology of seizures induced by sensitization with low intensity brain stimulation. *Pharmacol Biochem Behav* **1**: 461–467, 1973.
5. Blaschke, T. F. and K. L. Melmon. Antihypertensive agents and the drug therapy of hypertension. In: *The Pharmacological Basis of Therapeutics*, edited by A. Gilman, L. Goodman and A. Gilman. New York: McMillan Publishing Co., 1980, pp. 793–818.
6. Bowyer, J. F., T. E. Albertson and W. D. Winters. Cortical kindled seizures: Modification by excitant and depressant drugs. *Epilepsia* **24**: 356–367, 1983.
7. Corcoran, M. E. Catecholamines and kindling. In: *Kindling 2*, edited by J. A. Wada. New York: Raven Press, 1981, pp. 87–101.
8. Corcoran, M. E., H. C. Fibiger, J. A. McCaughran and J. A. Wada. Potentiation of amygdaloid kindling and metrazol-induced seizures by 6-hydroxydopamine in rats. *Exp Neurol* **45**: 118–133, 1974.
9. de Jong, R. H. *Local Anesthetics*, 2nd ed. Springfield, IL: Charles C. Thomas, 1977.
10. Gee, K. W., E. K. Killam and M. A. Hollinger. Effects of haloperidol-induced dopamine receptor supersensitivity on kindled seizure development. *J Pharmacol Exp Ther* **225**: 70–76, 1983.
11. Goddard, G. V., D. C. McIntyre and C. K. Leech. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* **25**: 295–330, 1969.
12. Joy, R. M., L. G. Stark and T. E. Albertson. Dose-dependent proconvulsant and anticonvulsant actions of the alpha-2 adrenergic agonist, xylazine, on kindled seizures in the rat. *Pharmacol Biochem Behav* **19**: 345–350, 1983.
13. Kalichman, M. W. Neurochemical correlates of the kindling model of epilepsy. *Neurosci Biobehav Rev* **6**: 165–181, 1982.
14. Knobloch, L. C., J. M. Goldstein and J. B. Malick. Effects of acute and subacute antidepressant treatment on kindled seizures in rats. *Pharmacol Biochem Behav* **17**: 461–465, 1982.

15. Langer, S. Z., M. S. Briley and R. Raisman. Regulation of neurotransmission through presynaptic receptors and other mechanisms: Possible clinical relevance and therapeutic potential. In: *Receptors for Neurotransmitters and Peptide Hormones*, edited by G. Pepeu, M. J. Kuhar and S. J. Enna. New York: Raven Press, 1980.
16. Lesse, H. Prolonged effects of cocaine on hippocampal activity. *Commun Psychopharmacol* **4:** 247–254, 1980.
17. Lesse, H. and J. P. Collins. Effects of cocaine on propagation of limbic seizure activity. *Pharmacol Biochem Behav* **11:** 689–694, 1979.
18. Lesse, H. and J. P. Collins. Differential effects of cocaine on limbic excitability. *Pharmacol Biochem Behav* **13:** 695–703, 1980.
19. Matsuzaki, M. and A. L. Misra. Cocaine and pseudococaine: Comparative effects on electrical after-discharge in the limbic system of cats. *Brain Res Bull* **3:** 341–347, 1978.
20. McIntyre, D. C., N. Edson, G. Chao and V. Knowles. Differential effect of acute vs chronic desmethylimipramine on the rate of amygdala kindling in rats. *Exp Neurol* **78:** 158–166, 1982.
21. McNamara, J. O., L. C. Rigsbee and M. T. Galloway. Evidence that substantia nigra is crucial to neural network of kindled seizures. *Eur J Pharmacol* **86:** 485–486, 1983.
22. Mohr, E. and M. E. Corcoran. Depletion of noradrenaline and amygdaloid kindling. *Exp Neurol* **72:** 507–511, 1981.
23. Pinel, J. P. J. and L. I. Rovner. Electrode placement and kindling-induced experimental epilepsy. *Exp Neurol* **58:** 335–346, 1978.
24. Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr Clin Neurophysiol* **32:** 281–294, 1972.
25. Racine, R., K. Livingston and A. Joaquin. Effects of procaine hydrochloride, diazepam, and diphenylhydantoin on seizure development in cortical and subcortical structures in rats. *Electroencephalogr Clin Neurophysiol* **38:** 355–365, 1975.
26. Racine, R., W. M. Burnham and K. Livingston. The effect of procaine hydrochloride and diazepam, separately or in combination, on cortico-generalized kindled seizures. *Electroencephalogr Clin Neurophysiol* **47:** 204–212, 1979.
27. Ritchie, J. M. and N. M. Greene. Local Anesthetics. In: *The Pharmacological Basis of Therapeutics*, edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: MacMillan Publishing Co., 1980, pp. 300–320.
28. Ross, S. B. and A. L. Renyi. Inhibition of the uptake of tritiated catecholamines by antidepressant and related agents. *Eur J Pharmacol* **2:** 181–186, 1967.
29. Ross, S. B. and A. L. Renyi. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. *Eur J Pharmacol* **7:** 270–277, 1969.
30. Segal, S. *Nonparametric Methods for the Behavioral Sciences*. New York: McGraw-Hill, 1956.
31. Stach, R., M. B. Lazarova and D. Kacz. The effects of antidepressant drugs on the seizures kindled from the rabbit amygdala. *Pol J Pharmacol Pharm* **32:** 505–512, 1980.
32. Stripling, J. S. Origin of cocaine- and lidocaine-induced spindle activity within the olfactory forebrain of the rat. *Electroencephalogr Clin Neurophysiol* **53:** 208–219, 1982.
33. Stripling, J. S. and C. Hendricks. Facilitation of kindling by convulsions induced by cocaine or lidocaine but not pentylenetetrazol. *Pharmacol Biochem Behav* **15:** 793–798, 1981.
34. Stripling, J. S. and C. Hendricks. Effect of cocaine and lidocaine on the expression of kindled seizures in the rat. *Pharmacol Biochem Behav* **14:** 397–403, 1981.
35. Stripling, J. S. and C. Hendricks. Effect of cocaine on afterdischarge threshold in previously kindled rats. *Pharmacol Biochem Behav* **16:** 855–857, 1982.
36. Stripling, J. S. and R. D. Russell. Effect of cocaine and pentylenetetrazol on cortical kindling. *Soc Neurosci Abstr* **8:** 458, 1982.
37. Westerberg, V., J. Lewis and M. E. Corcoran. Depletion of noradrenaline fails to affect kindled seizures. *Exp Neurol* **84:** 237–240, 1984.