Pharmacology Biochemistry & Behavior, Vol. 16, pp. 855–857, 1982. Printed in the U.S.A.

Effect of Cocaine on Afterdischarge Threshold in Previously Kindled Rats[1]

JEFFREY S. STRIPLING AND CURTIS HENDRICKS

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

Received 14 November 1981

STRIPLING, J. S. AND C. HENDRICKS. *Effect of cocaine on afterdischarge threshold in previously kindled rats.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 855–857, 1982.—Previous experiments have yielded conflicting reports on the effect of cocaine on the afterdischarge (AD) threshold for electrical stimulation. The present study was designed to determine if differences in the type of stimulation used could account for these discrepancies. Male Long-Evans rats which had been previously kindled by stimulation of the olfactory bulb were used to determine the AD threshold of the olfactory bulb following the intraperitoneal injection of saline or 20 mg/kg cocaine hydrochloride. AD's were elicited by trains of square-wave pulses which varied in frequency and train duration. Cocaine significantly increased the amount of current required to produce AD's using stimulus trains with frequencies of 30–100 pulses/sec, while evidence of a decrease in AD threshold by cocaine was found at a frequency of 20 pulses/sec. The results suggest that cocaine has opposite effects on AD threshold at high and low frequencies of stimulation.

Rat Afterdischarge threshold Olfactory bulb Cocaine

THERE is at present a lack of consensus in the literature concerning the effect of cocaine on the afterdischarge (AD) threshold for electrical stimulation at limbic sites. Lesse and Collins [1] reported that cocaine significantly reduced the AD threshold for stimulation of the hippocampus or amygdala (but not the septal area) in the cat, while Matsuzaki and Misra [2] found a significant elevation of AD threshold by cocaine or pseudococaine for stimulation of the same structures in the cat. In contrast to both of these findings, Stripling and Hendricks [3] found no significant effect of cocaine or lidocaine on the AD threshold for stimulation of the olfactory bulb in the rat (the prepyriform cortex and amygdala were also tested, but the AD threshold was too low in these sites to be measured accurately by the procedure used.).

The explanation for these conflicting results may lie in part in the type of stimulation used to elicit the AD. Lesse and Collins [1] found a decrease in AD threshold using long stimulus trains (up to 30 sec) of low-frequency square-wave pulses (3 pulses/sec). Matsuzaki and Misra [2], who found the opposite effect, also used long stimulus trains (10–20 sec) of square-wave pulses, but at a higher frequency (50 pulses/sec). This suggests that the discrepancy between these two experiments might be due to the difference in the frequency of stimulation. Stripling and Hendricks [3], who found no significant effect of cocaine on AD threshold, used the same pulse frequency as Matsuzaki and Misra [2] but a considerably shorter stimulus train (2 sec), suggesting that train duration might also influence the nature of the drug effect. The purpose of the present study was to evaluate these possibilities by determining the effect of cocaine on the current threshold for AD's elicited by stimulus trains varying in duration and pulse frequency.

METHOD

Subjects

The subjects were 39 male Long-Evans rats (Blue Spruce Farms) which weighed 460–660 g at the beginning of drug testing. They were housed individually in clear plastic cages and maintained on a 12-hr/12-hr light/dark cycle.

Procedure

All animals had previously been used as subjects in a study of the effect of repeated drug administration on the rate of kindling (Stripling, in preparation). They had been kindled by a 2-sec train of 0.2 msec negative square-wave pulses at a frequency of 50 pulses/sec, with the current level set at each animal's initial AD threshold, administered once per day via a monopolar 200-μm-diameter stainless-steel electrode implanted in the left olfactory bulb (9.1 mm anterior to bregma; 1.2 m lateral; 1.5 mm below the dura), with a stainless steel screw over the right anterior cortex used as a reference. All animals used in the present study had been in the saline control group or had received drug treatments which had not significantly affected the rate of kindling (9.7±0.4 AD's to criterion).

To assess the role of stimulation settings in cocaine's effect on AD threshold, two separate experiments using different animals were run. In Experiment 1 the train duration was held constant and the pulse frequency was varied, while in

[1]This research was supported by grants from the University Research Council of the University of Arkansas and the Marie Wilson Howells Fund. Address reprint requests to J. S. Stripling.

TABLE 1
EFFECT OF COCAINE ON AD THRESHOLD
(MEAN ± S.E.M.)

Stimulation Parameters		AD Threshold (μA)	
Frequency (Pulses/sec)	Train Duration (sec)	Saline	Cocaine
Experiment 1 (N=19)			
20	2	845 ± 5	726 ± 55*
30	2	337 ± 24	437 ± 43‡
40	2	203 ± 12	253 ± 14‡
50	2	153 ± 10	182 ± 11‡
100	2	113 ± 7	142 ± 9‡
Experiment 2 (N=20)			
30	2	460 ± 32	525 ± 44†
30	10	173 ± 15	214 ± 17‡

*At this stimulation setting, 14 of 19 animals exhibited no AD in either drug condition and were assigned a ceiling score of 850 μA. For the 5 animals which exhibited an AD in at least one drug condition, the AD threshold was significantly lower for cocaine (380 ± 107 μA) than for saline (830 ± 20 μA), $p<0.05$.
†$p<0.05$; ‡$p<0.01$.

Experiment 2 the pulse frequency was held constant and the train duration was varied.

The procedure for determination of AD threshold was similar in both experiments. An animal was injected intraperitoneally with either saline or 20 mg/kg cocaine hydrochloride and placed in a recording chamber enclosed in a larger chamber for visual and acoustic isolation. Eight min after injection the olfactory bulb was stimulated with a train of 0.2 msec negative monophasic square-wave pulses using a Grass S48 stimulator and PSIU6 stimulus isolation unit. If no AD occurred, the stimulation was repeated every 60 sec at increasing current levels until an AD was elicited.

The two experiments differed only in the stimulation settings used, which are summarized in Table 1 and described below:

Experiment 1. In this experiment the train duration was held constant at 2 sec and the animals were tested at 5 different frequencies of stimulation: 20, 30, 40, 50, and 100 pulses/sec. Each animal was tested once following saline and once following cocaine at each frequency setting for a total of 10 tests at 2-day intervals. The order of drug administration and stimulation setting was counterbalanced across animals. For each test, the current level for the initial train of pulses was set at 50 μA; this setting was increased by 50 μA for each subsequent stimulation until an AD was elicited. A maximum of 800 μA was administered; animals not exhibiting an AD by 800 μA were assigned a ceiling score of 850 μA.

Experiment 2. In this experiment the frequency of stimulation was held constant at 30 pulses/sec and the animals were tested once in each drug condition at each of 2 different train durations: 2 and 10 sec. For the 2-sec train the stimulation sequence was identical to that in Experiment 1. When a 10-sec train was used the current was increased in 25 μA increments in order to obtain finer resolution of the low AD thresholds obtained with the longer stimulation.

At the end of each experiment the electrode placements were verified histologically using the Prussian blue technique [3]. Due to non-homogeneity of variance across stimulation settings, the effect of cocaine on AD threshold was analyzed by a separate within-subjects analysis of variance for each stimulation setting.

RESULTS

The results are presented in Table 1. Cocaine produced a significant increase in AD threshold at both stimulation settings in Experiment 2 and at all but the lowest frequency setting in Experiment 1. The only stimulus setting at which there was a lower AD threshold for cocaine than for saline was the lowest frequency setting (20 pulses/sec) in Experiment 1. At this frequency setting, 14 animals did not exhibit an AD within the current range used and were assigned a ceiling score of 850 μA. Of the 5 remaining animals, 4 exhibited an AD only under cocaine and 1 under both drug conditions. For these 5 animals which responded to the stimulation with an AD in at least one drug condition, the AD threshold was significantly lower following cocaine (380±107 μA) than following saline (830±20 μA).

DISCUSSION

The outcome of Experiments 1 and 2 provides evidence for a frequency-dependent effect of cocaine upon AD threshold. At stimulation frequencies ranging from 30 to 100 pulses/sec, cocaine significantly elevated the AD threshold (Experiment 1); this effect was uninfluenced by substantial changes in the duration of the stimulus train (Experiment 2). In contrast, at the lowest frequency used in Experiment 1 (20 pulses/sec), cocaine significantly lowered the AD threshold in the 5 animals which exhibited an AD. It should be noted that at this frequency the majority of animals did not exhibit an AD in either drug condition; whether they would have exhibited a drug effect at higher current levels is a matter of conjecture. However, among the 5 animals that did respond the effect was quite large, underscoring the possibility that at low frequencies of stimulation cocaine's effect on AD threshold may in fact be the opposite of its effect at higher frequencies. More extensive evidence on this effect could not be collected due to the high AD threshold of the OB and its rapid rise at frequencies below 20 pulses/sec.

A frequency-dependent effect of cocaine would explain the apparent conflict between the findings of Matsuzaki and Misra [2], who found an elevation of AD threshold by cocaine using 50 pulses/sec stimulation, and Lesse and Collins [1], who found a decrease in AD threshold using 3 pulses/sec stimulation. In addition, the absence of a drug effect on AD threshold in the septal area found by Lesse and Collins [2] might be due not to an intrinsic difference in the action of cocaine on the septal area vs the other sites studied, but to the higher frequency of stimulation which had to be used in the septal area (9 pulses/sec) because of its higher AD threshold.

As previously indicated, Stripling and Hendricks [3] found no significant effect of cocaine or lidocaine on AD threshold in the olfactory bulb using a stimulation frequency of 50 pulses/sec. Upon reinspecting these data we found that the majority of the 11 animals with olfactory bulb electrodes in fact exhibited an elevation of AD threshold in the drug conditions, but that one animal exhibited a large response in the opposite direction. If this animal is omitted from the analysis, lidocaine produces a significant elevation of AD threshold, and the effect of cocaine approaches significance.

Thus it seems likely that the negative outcome in this previous study was due to the small sample size used and to an unusual response in one animal.

In summary, the outcome of the present experiments provides strong support for the elevation of AD threshold by cocaine at high frequencies, and statistically significant but less complete evidence for a decrease in AD threshold by cocaine at low frequencies. The implication that cocaine's effect on AD threshold can be reversed by altering the frequency of stimulation is a striking one which merits further investigation.

REFERENCES

1. Lesse, H. and J. P. Collins. Differential effects of cocaine on limbic excitability. *Pharmac. Biochem. Behav.* **13:** 695–703, 1980.
2. Matsuzaki, M. and A. L. Misra. Cocaine and pseudococaine: Comparative effects on electrical afterdischarge in the limbic system of cats. *Brain Res. Bull.* **3:** 341–347, 1978.
3. Stripling, J. S. and C. Hendricks. Effect of cocaine and lidocaine on the expression of kindled seizures in the rat. *Pharmac. Biochem. Behav.* **14:** 397–403, 1981.